VIRULENCE OF *SEPTORIA TRISETI* AND FUNGICIDE CONTROL OF LEAF MOTTLE AND FUSARIUM SEED INFECTION OF CANARY SEED (*PHALARIS CANARIENSIS*)

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ABSTRACT

Leaf mottle, caused by *Septoria triseti*, is the most important disease of canary seed (*Phalaris canariensis* L.) in western Canada and when severe it may cause reduction of canary seed yield. Understanding the host-pathogen interaction and the variation in virulence of the pathogen population is important for the development of durable resistance in canary seed cultivars. Recently, canary seed was approved as food for human consumption and identification of pathogenic fungal species on canary seed panicles is necessary to monitor seed quality. The objectives of this project were: 1) to evaluate variation for virulence among 27 isolates of *S. triseti* on *Phalaris* spp., 2) to identify the fungal species present on canary seed, and 3) to evaluate the effect of fungicides, application timings and canary seed genotypes on leaf mottle and fusarium seed infection of canary seed. Under controlled conditions, 24 *Phalaris* genotypes were evaluated for leaf mottle severity after inoculation with 27 isolates of *S. triseti* collected during 2005, 2013 or 2014. Differential interactions were detected in this study, which suggest that this patho-system follows the gene-for-gene model. Accession PI 189547 from Mexico was identified as resistant to 25 of the 27 isolates, which should be a valuable parent in a canary seed breeding program. Survey reports from 2014 and 2015 indicated the presence of *Alternaria* spp. and *Fusarium* spp. related to the FHB complex (*Fusarium graminearum* Schwabe, *F. culmorum* (W. G. Smith) Sacc., *F. avenaceum* (Corda ex Fr.) Sacc. and *F. poae* (Peck) Wollenw). A field study at Saskatoon and Indian Head during 2014 and 2015, using moderately resistant (PI 251274-3) and susceptible (Keet) canary seed genotypes, and three fungicides (propiconazole, prothioconazole + tebuconazole and pyraclostrobin + metconazole) applied at flag leaf and heading stages indicated that fungicide application reduced disease severity in years of high humidity, but application timing had little to no effect. Canary seed genotypes did not differ for leaf mottle severity or

fusarium seed infection. Although these studies increased our knowledge of the interaction between *S. triseti* and canary seed, the benefit of fungicide applications were more difficult to measure. Thus, more research is needed to integrate this information into effective strategies to control leaf mottle and FHB in this crop.

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DEDICATION

To my beloved father and mother, my daily inspiration to be a better person and professional, who taught me the value of work hard, dreaming big and to be happy.

TABLE OF CONTENTS

ABSTRACT ..... **ii**
ACKNOWLEDGEMENTS ..... **iv**
DEDICATION ..... **v**
TABLE OF CONTENTS ..... **vi**
LIST OF TABLE ..... **ix**
LIST OF FIGURES ..... **xi**
LIST OF ABREVATIONS ..... **xiii**
CHAPTER 1 : **1**
Introduction and research hypotheses **1**
1.1 Introduction 1
1.2 Hypotheses and objectives: 3
CHAPTER 2: **5**
Literature Review **5**
2.1 Canary seed (*Phalaris canariensis* L.) 5
2.1.1 Origin and classification 5
2.1.2 Distribution of canary seed 6
2.1.3 Cytological and morphological characteristics 6
2.1.4 Nutrient composition of canary seed and uses 7
2.1.1 Agronomic characteristics 9
2.2 Insect pests and diseases of canary seed 10
2.2.1 *Septoria triseti* Speg. 11
2.2.2 Host range of *Septoria triseti* 12
2.2.3 Distribution and symptoms of leaf mottle 12
2.2.4 Host-pathogen interactions 13
2.2.5 *Fusarium graminearum* Schwabe 15
2.3 Yield losses in canary seed 15
2.3.1 Yield loss caused by *Septoria* spp. 15
2.4 Fungicide control of *Septoria* spp. and FHB 16
2.4.1 Fungicide application timing to control *Septoria* spp. 16
2.4.2 Fungicide control of fusarium head blight 19

2.5 Summary 20
CHAPTER 3: 22
Variation for virulence of *Septoria triseti* on canary seed (*Phalaris canariensis*) under controlled conditions 22
3.1 Introduction 22
3.2 Material and Methods 23
3.2.1 Plant Material 23
3.2.2 *Septoria triseti* isolates 23
3.2.3 Inoculation of *S. triseti* 25
3.2.4 Disease assessment 26
3.3 Data analysis 27
3.4 Results 28
3.5 Discussion 31
3.6 Conclusion 34
CHAPTER 4. 35
Identification of fungal species on canary seed (*Phalaris canariensis*) in Saskatchewan. 35
4.1 Introduction 35
4.2 Material and Methods 36
4.2.1 Seed Material 36
4.2.2 Pathogenicity test on seeds 36
4.2.3 Koch's postulates for *Fusarium graminearum* on canary seed 37
4.3 Disease assessment and data analysis 37
4.4 Results 38
4.4.1 Fungal species on canary seed 38
4.4.2 *Fusarium* spp. in commercial canary seed crops in Saskatchewan in 2014 and 2015 39
4.4.3 *Fusarium graminearum* on canary seed in Saskatchewan 42
4.5 Discussion 42
4.6 Conclusion 44
CHAPTER 5: 45
Fungicide control of leaf mottle (*Septoria triseti*) and fusarium seed infection on canary seed (*Phalaris canariensis*) 45

5.1 Introduction 45
5.2 Material and Methods 46
5.2.1 Agronomical conditions 46
5.2.2 Treatments 47
5.2.1 Inoculation of *Septoria triseti* 49
5.2.2 Experimental design 49
5.2.3 Disease severity assessment on the field 49
5.2.4 Yield response and seed quality 50
5.3 Data analysis 50
5.4 Economic analysis 52
5.5 Results 52
5.5.1 Weather conditions 52
5.5.2 Fungicide treatments response 53
5.5.3 Effect of fungicide product, fungicide timing and genotype on canary seed diseases, grain yield and grain quality 55
5.5.4 Effect of three fungicides applied at the flag leaf stage on canary seed diseases, grain yield and grain quality 68
5.5.5 Effect of fungicide product applied at heading stage on canary seed diseases, grain yield and grain quality. 71
5.5.6 Benefit of single and multiple fungicide applications on canary seed diseases, grain yield and grain quality 78
5.5.7 Economic analysis of fungicide application on canary seed 85
5.6 Discussion 86
5.7 Conclusion 94
CHAPTER 6: 96
General discussion and future research 96
6.1 Discussion and conclusion 96
6.2 Future studies 97
REFERENCES 100
APPENDICES 114

LIST OF TABLE

Table 3.1 Identification and origin of 23 genotypes of *Phalaris canariensis* and one genotype of *P. brachystachys* (PI 380967) challenged with 27 isolates of *Septoria triseti* in this study. 24
Table 3.2 Isolates of *Septoria triseti* collected in 2007, 2013 and 2014 from commercial canary seed crops across Saskatchewan evaluated for disease reaction on *Phalaris* spp. genotypes in this study. 25
Table 3.3 Scale used to evaluate symptoms of *Septoria triseti* on 24 genotypes of canary seed under controlled conditions 27
Table 3.4 Susceptible (S) and resistant (R) responses caused by 27 *Septoria triseti* isolates collected from canary seed crops in Saskatchewan in 2007, 2013 and 2014, among 23 genotypes of *Phalaris canariensis* (canary seed) and one genotype of *Phalaris brachystachys* (PI380967). 30
Table 4.1 Incidence (%) of fungal species identified on canary seed kernels from fungicide untreated plots at Saskatoon and Indian Head, 2014 and 2015. 39
Table 4.2 Prevalence of *Fusarium* spp. in commercial fields, and incidence on 100 kernels of each crop in Saskatchewan in 2014 and 2015 40
Table 4.3 Prevalence (% of crops) of *Fusarium* spp. in crop districts in Saskatchewan in 2014 (21 crops) and 2015 (26 crops). 41
Table 5.1 Fungicide application timing treatments to control leaf mottle and fusarium seed infection on two canary seed genotypes at Saskatoon and Indian Head, Saskatchewan in 2014 and 2015 48
Table 5.2 Rating scale used to evaluate leaf mottle severity on canary seed under field conditions. 50
Table 5.3 Minimum, maximum, and mean monthly temperature (^{o}C), and precipitation (mm) at Saskatoon and Indian Head, Saskatchewan, from May to August, 2014 and 2015. 53
Table 5.4 Summary of means of fourteen treatments on leaf mottle disease severity, fusarium seed infection, yield, TKW, protein content and oil content of canary seed at Indian Head and Saskatoon in 2014 and 2015 54
Table 5.5 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015. 55
Table 5.6 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on fusarium seed infection (%) at Indian Head and Saskatoon in 2014 and 2015..... 58
Table 5.7 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on yield (kg ha^{-1}) at Indian Head and Saskatoon in 2014 and 2015..... 60
Table 5.8 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI

251274-3) on grain quality traits on canary seed at Indian Head and Saskatoon in 2014 and 2015. 61
Table 5.12 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015. 71
Table 5.13 Probability of *F* values for the analysis of variance for fungicide and genotype on fusarium seed infection (%) at Indian Head and Saskatoon, 2014 and 2015. 72
Table 5.14 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on yield (kg ha^{-}1) at Indian Head and Saskatoon, 2014 and 2015. 73
Table 5.15 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on TKW (g) at Indian Head and Saskatoon, 2014 and 2015..... 74
Table 5.16 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on protein (%) at Indian Head and Saskatoon, 2014 and 2015. 75
Table 5.17 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on oil (%) at Indian Head and Saskatoon, 2014 and 2015..... 77
Table 5.18 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015. 79
Table 5.19 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follow by propiconazole on fusarium seed infection (%) at Indian Head and Saskatoon, 2014 and 2015. 80
Table 5.20 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on yield (kg ha^{-1}) at Indian Head and Saskatoon, 2014 and 2015. 81
Table 5.21 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on TKW (g) at Indian Head and Saskatoon, 2014 and 2015. 82
Table 5.22 Probability of *F* values for the frequency of fungicide applications: pyraclostrobin + metconazole or pyraclostrobin + metconazole follow by propiconazole on protein content in canary seed at Indian Head and Saskatoon, 2014 and 2015. 83
Table 5.23 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on oil (%) at Indian Head and Saskatoon, 2014 and 2015. 84
Table 5.24 Net return of fungicide application at leaf stage to control leaf mottle on canary seed at Indian Head 2014. 86

LIST OF FIGURES

Fig. 4.1 Fungal species present on canary seed. Average of two years: 2014 and 2015 samples. 38
Fig. 4.2 Map of Saskatchewan with crop districts; circles indicate general areas from where canary seed samples were obtained 41
Fig. 5.1 Effect of propiconazole and prothioconazole + tebuconazole on leaf mottle disease severity (%) at Indian Head in 2014 55
Fig. 5.2 Leaf mottle severity of canary seed genotypes ($P \leq 0.05$) Keet and PI 251274-3 at Indian Head in 2014.. 56
Fig. 5.3 Interaction of variety and fungicide timing effects, on control of leaf mottle of canary seed at Indian Head and Saskatoon in 2015. 57
Fig. 5.4 Fusarium seed infection on canary seed after application of propiconazole or prothioconazole + tebuconazole at Indian Head in 2014.. 58
Fig. 5.5 Effect of application timing on fusarium seed infection at Indian Head and Saskatoon in 2014. 59
Fig. 5.6 Yield of two canary seed genotypes Keet and PI 251274-3 at Saskatoon 2014 and Indian Head in 2015.. 60
Fig. 5.7 Interaction of genotypes and timing on yield of canary seed at Saskatoon in 2015 61
Fig. 5.8 Thousand Kernel Weight (g) of two canary seed genotypes (PI 251274-3 and Keet) at Saskatoon and Indian Head in 2014 and 2015. 62
Fig. 5.9 Interaction of timing and genotype on thousand kernel weight on canary seed at Saskatoon in 2015. 62
Fig. 5.10 Protein content of canary seed genotypes, Keet and PI 251274-3 at Saskatoon and Indian Head in 2014 and 2015. 63
Fig. 5.11 Interaction of fungicide and timing on oil content of canary seed genotypes Keet and PI 251274-3 at Indian Head in 2014. 65
Fig. 5.12 Interaction of timing and genotype on oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon in 2014. 66
Fig. 5.13 Interaction of fungicide product, fungicide application timing, and genotype, A: propiconazole and B: prothioconazole + tebuconazole, on oil content on canary seed at Indian Head in 2015. 67
Fig. 5.14 Oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon in 2015.. ... 67
Fig. 5.15 Leaf mottle severity of canary seed genotypes Keet and PI 251274-3 at Indian Head in 2015. 71
Fig. 5.16 Fusarium seed infection on canary seed after application of propiconazole and prothioconazole + tebuconazole at Saskatoon, 2014.. 72
Fig. 5.17 Grain Yield (kg ha^{-1}) of two genotypes of canary seed after application of propiconazole or prothioconazole + tebuconazole at Indian Head. 73
Fig. 5.18 Thousand Kernel Weight (g) of two canary seed genotypes (Keet and PI 251274-3) to control leaf mottle at Saskatoon and Indian Head in 2014 and 2015. 74

Fig. 5.19 Protein content of canary seed genotypes, Keet and PI 251274-3 after application of propiconazole and prothioconazole + tebuconazole at Saskatoon and Indian Head in 2014 and 2015..... 76
Fig. 5.20 Protein content of canary seed genotypes after application of propiconazole or prothioconazole + metconazole at Saskatoon 2014. 76
Fig. 5. 21 Oil content after application of two fungicides on canary seed at Indian Head in 2014……77
Fig. 5.22 Oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon and Indian Head in 2015. 78
Fig. 5. 1 Interaction of two factors, fungicide and genotype, to control leaf mottle on canary seed at Indian Head ($P \leq 0.05$)…….79
Fig. 5. 24 Frequency one or two fungicide applications to control leaf mottle on canary seed at Saskatoon in 2015.. 79
Fig. 5.25 Effect of fungicide frequency, one application: pyraclostrobin + metconazole and two applications: pyraclostrobin + metconazole follow by propiconazole on yield of canary seed at Indian Head in 2014. 81
Fig. 5.26 Effect of fungicide frequency, one application: pyraclostrobin + metconazole and two applications: pyraclostrobin + metconazole follow by propiconazole on yield of two genotypes of canary seed at Indian Head in 2015. 82
Fig. 5.27 Frequency of fungicide application and genotype on TKW of canary seed at Indian Head and Saskatoon in 2014 and 2015.. 83
Fig. 5.28 Effect of genotype on oil content in canary seed at Indian Head and Saskatoon in 2014 and 2015.. 84

LIST OF ABREVATIONS

Speg.: Spegazzini

FRAC: Fungicide Resistance Action Committee

BBCH: Biologische Bundesanstalt, Bundessortenamt and Chemical industry

FHB: fusarium head blight

CDC: Crop Development Centre

R: resistance

Avr: avirulence

HR: hypersensitive response

DON: deoxynivalenol

NIV: nivalenol

GS: growth stage

t ha^{-1} : tonnes per hectare

STB: septoria tritici blotch

FDK: fusarium damaged kernels

LM: leaf mottle

YMA: yeast media agar

HR: relative humidity

dai: days after inoculation

TKW: thousand kernel weight

lat.: latitude

long: longitude

CHAPTER 1 :

Introduction and research hypotheses

1.1 Introduction

Canary seed (*Phalaris canariensis* L.) is an annual grass that belongs to *Poaceae* family. It is used primarily to feed caged birds, although recently it was approved as food for human consumption. Canada is the largest producer of canary seed with an annual seeded area of approximately 113717 ha during the past five years (Statistics Canada, 2016). Saskatchewan canary seed growers are responsible for approximately 90% of the Canadian production (Saskatchewan Ministry of Agriculture, 2014). One major reason for reduced canary seed yield is the occurrence of leaf mottle, caused by *Septoria triseti* Speg., which reduces the green leaf area and therefore, photosynthesis (Blandino and Reyneri, 2009). In 1988, leaf mottle was the most widespread and severe disease of canary seed in Saskatchewan, surpassing root rot and spot blotch (Berkenkamp et al., 1989). Understanding the host-pathogen interaction, such as the variation in virulence of a pathogen population, is important for the development of durable resistance in canary seed cultivars. However, little information is known about the host-pathogen interaction between *S. triseti* and *P. canariensis*.

Fungicides are one of the most common strategies used by farmers to control crop diseases known to reduce grain yield and quality. To control wheat leaf diseases such as *S. tritici* (Rob. Ex Desm), *S. nodorum* (Berk.) and *Pyrenophora tritici-repentis* (Died.) Drechsler, the triazole group of fungicides (Group 3) has been used. These fungicides directly affect the biosynthesis of sterols

(FRAC code list, 2013) by blocking the C14-demethylase enzyme. Sterols are necessary for cell membrane formation. When sterols are affected by fungicide, the fungal cell membrane and cell division and growth is affected, resulting in morphological changes and reduction of fungal growth (Yoshiyuki et al., 2013). Leaf mottle of canary seed is controlled by propiconazole in western Canada (Saskatchewan Ministry of Agriculture, 2015). In some crops, mixtures of fungicides from more than one group, or rotation of products from two or more groups, such as pyraclostrobin (Group 11) and metconazole (Group 3) are used to control a broad range of pathogens in crops. One application of prothioconazole + tebuconazole applied between BBCH (Lancashire et al. 1991) growth stages 60 and 80 (flowering and ripening) was able to reduce *Zymoseptoria tritici* Rob ex Desm. severity by 50% and increase yield by 20% in wheat (Rodrigo et al., 2014). In canary seed, yield increases of 20 - 40% have been observed after application of fungicides to reduce leaf mottle severity in the crop (May et al., 2000). It is essential to identify appropriate fungicide application timing to protect the crop and yield; and it is necessary to assess the effectiveness of fungicides to control *S. triseti* on susceptible and moderately resistance genotypes of canary seed under field conditions.

Seed infection of species of the genera *Alternaria* and *Fusarium* are common in infected seed from the field. *Fusarium graminearum* Schwabe, *F. culmorum* (Wm. G. Smith) Sacc, *F. avenaceum* (Corda ex Fr.) Sacc. and *F. poae* (Peck) Wollenw, are the main species associated with fusarium head blight (FHB) (Parry et al., 1995). Survey reports from 2013, 2014 and 2015 indicated the presence of *F. graminearum* on canary seed (Vera et al. 2014, Cholango-Martinez et al., 2015). Fungal infection of seed in the field may affect the yield and quality of canary seed.

1.2 Hypotheses and objectives:

This project was composed of three studies, for which the hypotheses and objectives were:

Study 1:

Hypothesis

- Differential interactions exist in the *Phalaris canariensis* - *Septoria triseti* host-pathogen system.

Objective

- To evaluate variation of virulence among 27 isolates of *Septoria triseti* on 23 genotypes of *Phalaris canariensis* and one genotype of *P. brachystachys*.

Study 2:

Hypothesis

- *Fusarium graminearum* infects canary seed under field conditions.

Objective

- To identify the *Fusarium* spp. and other fungal species on canary seed seeds.

Study 3:

Hypotheses

- Fungicide application at heading stage is more effective than at flag leaf stage to reduce leaf mottle and fusarium seed infection disease severity on susceptible canary seed genotypes.

- Two fungicide applications are more effective than a single application to reduce leaf mottle and fusarium seed infection of canary seed.

Objectives

- To evaluate the effect of fungicide products (propiconazole, prothioconazole + tebuconazole, pyraclostrobin + metconazole), fungicide application timings (flag leaf and heading stages) and canary seed genotypes (PI 251274-3 and Keet) on leaf mottle and fusarium seed infection.

CHAPTER 2:

Literature Review

2.1 Canary seed (*Phalaris canariensis* L.)

2.1.1 Origin and classification

Canary seed (*Phalaris canariensis* L.) is an annual grass that originated in the Canary Islands and was first domesticated in the Mediterranean region (Anderson, 1961). Although there is evidence that canary seed was used in flour blends for making bread, there is no indication of where canary seed was domesticated (Körnicke and Weber, 1885). In North America, the production of canary seed began in Minnesota and North Dakota, USA after World War II. In western Canada, canary seed was first produced in the 1970s and 1980s (Agri-Facts, 1998).

Canary seed belongs to the order Poales, family Poaceae, sub-family Pooideae, and genus *Phalaris*. The *Phalaris* genus includes 22 species, such as *P. brachystachys*, *P. paradoxa*, *P. minor*, *P. arundinaceae* and *P. canariensis* (Baldini, 1995). Although some species of this genus (*P. minor*, *P. brachystachys* and *P. paradoxa*) have been reported as weeds in Pakistan, India, the Mediterranean basin and Australia; other species such as *P. arundinaceae* (forage crop), *P. angusta* (fodder crop) and *P. canariensis* (grain crop for birds) are used as feed animal. *Phalaris canariensis* is the only member of this genus that retains ripe seeds in the panicles after maturity (Baldini, 1995) facilitating cultivation and harvest. Although canary seed belongs to the Poacea (Graminae) family and sub-family Pooideae, it is genetically related to cereals such as oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Li et al., 1997). Annual

canary seed has similar maturity to wheat and production practices are also similar (Robinson, 1978). *Phalaris canariensis* is believed to be the cultivated form of *P. brachystachys* as a result of a single dominant to recessive mutation (Oram, 2004). That study clarified the relationship between wild and domesticated taxa and provided evidence that *P. canariensis* and *P. brachystachys* belong to the same biological species, making *P. brachystachys* the wild ancestor of *P. canariensis.* The wild relatives of some species have been reported to be valuable sources of genetic resistance to several diseases, for example *Septoria* complex, was detected in wild relatives of wheat (Yechilevich-Auster et al., 1983), the resistance gene (5D chromosome) present in *Aegilops squarrosa* confers resistance against *Septoria nodorum* in seedlings of wheat (Nicholson et al., 1993).

2.1.2 Distribution of canary seed

Phalaris canariensis is cultivated in many countries with temperate climates. Currently, production is concentrated in the western provinces of Canada (approximately 131,000 t annually) and on a smaller scale in Argentina (52,900 t), Thailand (34,400 t) and Australia (5.6 t) and Hungary (5 t) (FAOSTAT, 2013).

2.1.3 Cytological and morphological characteristics

Annual canary seed is a self-pollinated diploid, with an upper limit of open pollination of 2.2% (Matus-Cadiz and Hucl, 2006); it has 2n = 12 chromosomes and a genome of 3,800 Mbp (Li etal., 2011). Canary seed is an herbaceous grass with a shallow root system; thus, it is sensitive to dry conditions (McVicar et al., 2002). It has the typical morphological structure of a grass; its height is approximately 60-115 cm, with many tillers and an erect growth habit. The ligules are obtuse and approximately 6 - 8 mm long, and the leaf blades 20 - 40 mm long by 5 - 10 mm wide. The flowers are arranged on oval-shaped panicle that contains approximately 200 florets depending on

the variety. *Phalaris canariensis* can be differentiated from other species in the Phalaris genus by the large sterile florets, which are between 4.8 - 6.8 mm (Anderson, 1961; Matus, 1996). The mature fruit consists of a fertile floret and two reduced sterile basal florets. The length of the groat is 3.9-4.2 mm and width 1.4-1.7 mm (Matus, 1996), with an elliptical shape covered by hulls. Canary seed hulls are covered by microscopic hairs (trichomes) composed of 98% silica. This makes canary seed difficult to work with because it causes skin irritation (Putman et al. 1996). The glabrous characteristic of canary seed has been identified to be controlled by a single gene (Matus-Cadiz et al., 2003), with the glabrous phenotype recessive to the pubescence condition. Based on the presence or absence of trichomes, canary seed cultivars are either hairy or hairless. The hairy cultivars include Keet (Robinson, 1979) and Elias (Robinson, 1983), and the glabrous or hairless type includes CDC Maria (Hucl, 1997), CDC Togo and CDC Bastia.

2.1.4 Nutrient composition of canary seed and uses

Canary seed is used mainly in bird feed mixes for caged and wild birds. Since *P. canariensis* has a high level of protein, oil and starch, some research has been conducted to investigate the use of canary seed as a potential food crop for human consumption and animal feed, as well as for industrial uses. Canary seed (18-21%) has higher protein content than other cereals such as: barley (10-17%), oat (13%) and wheat (8.5-15%) (Gutierrez-Alamo et al., 2008; Quinde et al., 2004). Proteins are some of the most important nutrients for the human body and need to be included as a part of the daily diet, thus consumption of canary seed as a food may be a new source of protein. Also, Robison (1978) reported that canary seed has 19% amino acid concentration in the caryopses, which places canary seed in a group with many pulse and oilseed crops.

The crude fat in canary seed groats (8.7%) have five times more lipid content than wheat and is composed of linoleic (55%), oleic (29%), palmitic (11%) and linoleic (2.5%) acids (Malik and

Williams, 1996). As in most cereals, crude fat is present in higher concentration in the bran fraction than in the flour fraction. The crude fat content of canary seed whole grain flour (7.7 - 8.0%) is similar to that of oats (7.5%), as reported by Kirk and Sawyer (1999), much higher (1.5 - 2.4%) than for wheat (Gutierrez-Alamo et al., 2008) or for barley (1 - 2%) (Quinde et al., 2004). The high crude fat content in canary seed may be beneficial as a functional food ingredient due to its antioxidant properties and low concentration of saturated fat (Abdel- Aal et al., 1997). In addition, canary seed has high levels of carotenoids and phenolics, making it useful as a food ingredient with potential health properties.

The canary seed groats are composed of starch granules and protein bodies embedded in a protein matrix similar to that of the oat kernel. The starch content in canary seed groats is 61% and the starch grain size is 2.0 μm. Kernel size is an important characteristic for digestibility since the smaller starch size, combined with the amount of amylose make the grain highly digestible (Abdel-Aal and Hucl 2005). In addition, starch and amylose content affect the baking process; baking tests have shown that bread made with 100% hairless canary seed flour was significantly lower in loaf volume and crust and crumb color than was bread made with wheat flour. However, using up to 25% hairless canary seed or 15% roasted canary seed flour it is possible to achieve a loaf volume and crust color comparable to wheat bread, demonstrating its potential for food applications.

Canary seed has been tested as animal feed for pigs and chickens. Thacker (2003) compared three pig diets: barley, soybean and canary seed and reported that dry matter digestibility decreased by increasing canary seed content, which replaced barley. In contrast, crude protein digestibility increased linearly, similar to barley and soybean diets, which indicates that canary seed can be successfully fed to growing-finishing pigs without dramatically affecting pig performance or carcass characteristics. In chicken diets, Newkirk et al. (2011) studied the effect of canary seed

on fed broiler chickens to evaluate nutrient value and possible toxicity. They concluded that canary seed does not affect chicken health nor affect broiler performance. Classen et al. (2014) examined the effects of dietary levels (0, 15, 30 and 45%) of hulled yellow (C05041) and brown (CDC Maria) canary seed on the performance and health of broiler chickens. They reported that growth rate and feed intake were affected in a quadratic manner by the amount of canary seed from 0 to 21 days, with the highest growth achieved by diets that included 15 and 30% canary seed. There was no effect of including canary seed in treatments between 22 to 35 days. Feed to gain ratio decreased linearly with increasing canary seed content for 0 to 21 day and 22 to 35 day, time periods. Mortality was not affected by canary seed content. The treatment did not affect gross necropsy at the trial end or histopathology of key organs. The conclusion of these studies indicates that yellow and brown hairless canary seed are beneficial and safe as poultry feed.

2.1.1 Agronomic characteristics

The life cycle of canary seed is approximately 114 days, and it varies by variety, for example, Cantate is 103 days, CDC Maria, 101 days and CDC Togo, 102 days (Saskatchewan Ministry of Agriculture). It is recommended this crop be grown in clay soils rather than in sandy soils due to sensitivity to drought. Canary seed has a significant yield response to seeding date. In Saskatchewan, canary seed is recommended to be seeded between early or mid-May (May et al., 2001). Delaying seeding from the early (30 April - 4 May) to the late date (29 to 30 May) reduced canary seed yield by 29% and panicle density by 24% (Miller, 2000).

Seeding rate has a limited effect on grain yield of canary seed. Yield response is minimal at seeding rates of 35 to 45 kg ha^{-1}, although grain yield tends to decrease as the seeding rate increases (May et al. 2012). The recommended seeding rate is 30-38 kg ha^{-1} with expected yields of 784 - 1,176 kg ha^{-1} (Saskatchewan Ministry of Agriculture, 2014).

The nitrogen and phosphorus requirement for canary seed varies among fields and soil types, the general recommendation in Saskatchewan is: 39 kg ha^{-1} N and 33 kg ha^{-1} P (Saskatchewan Ministry of Agriculture, 2014). The greatest increase in yield of canary seed after application of five nitrogen rates (20, 40, 60, 80 and 100 kg ha^{-1}) was between 20 and 40 kg N ha^{-1}, with a 2.3 kg ha^{-1} increase in grain yield for each kg of N fertilizer (May et al., 2012). There was a slight increase in grain yield as the nitrogen rate increased above 40 kg ha^{-1}, but the variability in grain yield also increased, reducing the incentive for growers to use N rates above 40 kg ha^{-1}.

Weed control in canary seed is important since *P. canariensis* is a poor competitor due to its low seedling vigor and slow growth rate between emergence and tillering (Putman et al., 1996). Holt and Hunter (1987) suggested use of bromoxynil, bromoxynil plus MCPA, linuron plus MCPA and propanil plus MCPA for control of broadleaf weeds in canary seed, as the crop has excellent tolerance to these products. Grassy weeds were difficult to control because there is a narrow margin of selectivity. In Saskatchewan, eight herbicides are register to control weeds in canary seed: Avadex ® (8-triallate), Avenge ® (8-difenzoquat), Bromoxynil ® (6-bromoxynil), Bromoxynil/MCPA ® (6-bromoxynil/4-MCPA), Curtail M (4-clopyralid & MCPA) ®, Dicamba + MCPA ® (4-dicamba & MCPA), Dicamba/Mecoprop/MCPA ® (4-dicamba, mecrop-p & MCPA), Prestige XC ® (4-fluroxypyr, clopyralid & MCPA) and Trophy® (4-fluroxypyr & MCPA) (Saskatchewan Ministry of Agriculture, 2015).

2.2 Insect pests and diseases of canary seed

A number of insects have been observed and reported in canary seed. In dry years, these include the English grain aphid (*Macrosiphum avenae* (Fabr.)) and the oat bird cherry aphid (*Rhopalosiphum padi* (L.)) (Saskatchewan Ministry of Agriculture, 2014).

A number of diseases are observed in countries where canary seed is cultivated. In Canada, diseases reported are: leaf mottle, (*Septoria triseti*, Berkenkamp et al., 1989), anthracnose (*Colletotrichum graminicola Ces. Wils.*, Holzgang and Pearse, 2009), common root rot (*Cochliobolus sativus* Ito & Kurib., *Fusarium* spp., Holzgang and Pearse, 2010), ergot (*Claviceps purpurea* (Fr.) Tul.)), and spot blotch (*Cochliobolus sativus* Ito & Kurib., Holzgang and Pearse 2011). In Argentina, alternaria (*Alternaria* spp), ergot (*Claviceps purpurea* Ito & Kurib.), seedling blight caused by *Fusarium* spp. and *Gibberella* spp. (*Gibberella gordonii*, *Giberella intricans* and *Giberella zeae*), magnaporthe grey leaf spot (*Magnaporthe grisea*), rust (*Puccinia graminis*), scald (*Rhynchosporium secalis*), septoria (*Septoria macrostoma*) and rhizoctonia (*Thanatephorus cucumeris*) (Pedraza and Perez, 2010). In Australia, the only disease noted on commercial canary seed experiments conducted in Queensland was powdery mildew (*Erysiphe graminis*) (Norton and Ford, 2002).

2.2.1 *Septoria triseti* Speg.

The taxonomical classification of *Septoria triseti*, the agent causal of leaf mottle in canary seed, is: phylum Ascomycota, class Dothideomycetes, order Capnodiales, family Mycosphaerellaceae and genus *Septoria* (Spegazzini, 1888) or *Zymoseptoria* (new classification for *Septoria* genus). It was isolated for the first time from *Agrostis magellanica* Lam. samples collected in southern Argentina (Sprague, 1960).

Septoria triseti conidiomata are pycnidial, sub epidermal, dark brown, sub globose, and 40 - 95 µm (Berkenkamp et al., 1989). Conidia are hyaline, filiform, and straight or slightly curved 17 - 34 x 1.4 - 2.2 µm, and aseptate or uniseptate. Microconidia were reported to be produced occasionally in the same conidiomata as with conidia or in separate spermagonia; they are hyaline, aseptate, filiform, and 5.5 - 9.6 x 0.7 - 1.0 µm (Sprague, 1960; Berkenkamp et al., 1989).

2.2.2 Host range of *Septoria triseti*

Various species of grasses have been reported as hosts of *S. triseti*: red top (*Agrostis alba* L.), highland bentgrass (*A. castellana* Boiss. & Reuter), spike bent (*A. exarata* L.), spike redtop or western bentgrass (*A. exarata* var. *ampla*), black bentgrass (*A. gigantean* Roth.) (Conners, 1967; Ginns, 1986), creeping bentgrass (*A. stolonifera* L.), browntop colonial bent or colonial bentgrass (*A. tenuis* Sibth.), annual junegrass (*Koeleria phleoides*) (Sprague, 1960), canary seed (*P. canariensis* L.) (Berkenkamp et al., 1989), and lesser canarygrass (*P. minor* Retz.) (Fatehi et al., 1993). There is no evidence that this fungus is present in other cereal crops.

2.2.3 Distribution and symptoms of leaf mottle

The development of the disease is related to favorable environment conditions and host pathogen interactions. Similar to other leaf diseases in cereals, such as septoria tritici blotch, stagonospora nodorum blotch and tan spot, leaf mottle of canary seed is considered a residue-borne disease (Saskatchewan Ministry of Agriculture, 2014). When canary seed is sown on, or adjacent to, canary seed stubble, canary seed has a higher risk of developing leaf mottle (McVicar et al., 2002). This disease is observed in the northwestern United States, Argentina (Sprague, 1960) and Canada (Berkenkamp et al., 1989) under wet and temperate conditions. In Canada, the first report of leaf mottle was in three of five fields surveyed (60%) in northeast Saskatchewan (Berkenkamp and Kirkham, 1989). Vera et al. (2014) reported the presence of leaf mottle to be 81% among 26 fields evaluated in 2013, which were located in northeast, west-central and southeast Saskatchewan and Cholango-Martinez et al. (2015) reported disease prevalence of leaf mottle to be 71% among 21 fields surveyed during the summer of 2014 in southern Saskatchewan.

The symptoms of leaf mottle appear first on the bottom of canary see leaves as pale tan to gray, oval lesions with diffuse margins on leaf blades and sheaths, although the early symptoms are difficult to recognize (Saskatchewan Ministry of Agriculture, 2014). Disease symptoms begin as indeterminate and irregular lesions, present on the tips of the leaves (Sprague, 1960). In these lesions, numerous small, brown pycnidia are formed and the distal portion of the leaf tissue is dead above large lesions (Berkenkamp et al., 1989). Under wet conditions, pycnidia ooze golden brown globs of spores that spread to healthy leaves by rain splash (Saskatchewan Ministry of Agriculture, 2014).

2.2.4 Host-pathogen interactions

Plants have an innate ability to recognize potential pathogens on the leaf surface and to resist infection. Susceptibility or resistance of plants is not only specific to the species of pathogen, but also to the specific genotype of the pathogen. Pathogen isolates to which the host resistance response is effective are considered avirulent, and isolates to which the host resistance response is not effective are considered virulent. When plant genotypes are challenged with a number of isolates, a differential response spectrum may be identified. These differences in virulence may be due to specific genes for resistance in the host plant. Pathogen variation among a number of host lines is believed to be due to the gene-for-gene interaction between host resistance genes and pathogen avirulence genes (Flor, 1971). A host that has a resistance (R) gene may possess alternative alleles that interact with a corresponding specific avirulence (Avr) gene in the pathogen, which also may have alternative alleles. This interaction pattern is the basis for biochemical investigations and for plant breeding for disease resistance. Van der Plank (1963) proposed that resistance be classified into two types: vertical resistance that is effective only against certain races, thus a gene-for-gene interaction occurs; and horizontal resistance, which is effective against all

races and outside the gene-for-gene system. Qualitative resistance is conferred by an R-gene or genes, according to the gene-for-gene model, and when effector-triggered immunity is activated, it results in the hypersensitive response (HR). The other kind of resistance is call general or quantitative, and is assumed to be polygenic and evaluated in a quantitative manner, as slower development of the disease resulting in reduced infection efficiency, less sporulation and a longer latent period (Van der Plank, 1963). Characterization of the host and the pathogen identifies isolate-specific or non-isolate specific reactions within a pathosystem (Parlevliet, 1993). Significant isolate-cultivar interactions are an indication of specific virulence in the pathosystem and it provides insight into the resistance genes (Van der Plank, 1968).

Significant interactions between cultivars of wheat and *Septoria* isolates indicate the presence of specific virulence and resistance, indicating a gene-for-gene system (Eyal and Levy, 1987; Kema and Van-Silfhout, 1997). Each wheat resistance gene has a corresponding specific avirulence gene in *S. tritici* (Branding et al., 2002). The virulence pattern observed from 74 isolates of *M. graminicola* collected in western Canada on six wheat genotypes indicated great physiological variation (Grieger et al., 2005).

Significant interactions between wheat and *S. nodorum* suggest a gene-for-gene interaction (Ali and Adhikari, 2008), also McCartney et al. (2002) studied the inheritance of resistance in intraspecific reciprocal crosses between hexaploid wheat lines Salamouni, ST6, Katepwa, and Eric, and the durum wheat lines Coulter and 4B1149 to two isolates of *M. graminicola* under controlled conditions. They reported that resistance was controlled by incompletely dominant genes in all cases; this indicated that isolate-specific resistance of wheat to *M. graminicola* follows a gene-for-gene model.

2.2.5 *Fusarium graminearum* Schwabe

Fusarium graminearum Schwabe is the most dominant, widespread and destructive pathogen of wheat in growing areas that have humid to semi-humid climates. Fusarium head blight (FHB) is a destructive disease of wheat, barley and other cereals caused by *Fusarium* spp. (Parry et al., 1995; McMullen et al., 1997; Liddell et al., 2003). Under conditions favorable for the development of FHB, grain yield and test weight may be reduced. The grain affected by FHB may become contaminated with deoxynivalenol (DON) or nivalenol (NIV) mycotoxins (Parry et al., 1995). Fusarium head blight causes yield loss due to the premature senescence of the panicle and reduces the quality of the grain due to the mycotoxins that form in the grain (Del Ponte et al., 2007). Although there are anecdotal reports of fusarium infected canary seed kernels, no studies exist on the impact of *F. graminearum* on canary seed under field conditions. In addition, other species of *Fusarium* spp. reported to cause FHB on cereals, have been detected on canary seed, such as *F. culmorum* (W. G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc., and *F. poae* (Peck) Wollenw (Cholango-Martinez, 2015).

2.3 Yield losses in canary seed

2.3.1 Yield loss caused by *Septoria* spp.

There are many regions in the world where *Septoria* spp. are serious pathogens of wheat, one of the crops on which *Zymoseptoria* spp. are reported to cause significant yield losses due to leaf spotting as a result of a reduction in solar interception of the flag leaf and spike (Scharen and Taylor, 1968; Krupinsky et al., 1973; Gaunt, 1995). Waggoner and Berger (1987) reported a strong relationship between yield and solar interception or green leaf area. When this relationship is weak, the amount of carbohydrates accumulated during grain filling decreases, causing yield

reduction at crop maturity (Eyal, 1999). During grain filling, assimilate availability comes from various sources: photosynthesis in healthy areas and water soluble carbohydrates stored in the stems are translocated to the grain (Ehdaie et al., 2008; Bingham et al., 2009). This occurs mainly on the upper three leaves (Thomas et al., 1989) and the risk of yield loss is greatest when the flag and penultimate leaves become severely infected early in the growing season (El Jarroudi et al., 2009). In Western Europe, septoria tritici blotch was reported to induce up to 30 - 40% yield loss when the upper leaves are severely infected (Eyal et al., 1987), and crop losses of 10 - 25% have been reported in Romania (Gheorghies, 1978).

Septoria nodorum was reported to cause yield losses in wheat up to 18% in fungicide experiments in Romania (Schluter and Janati, 1976), and between 25 - 30% in regions of high rainfall, such as Germany (Obst and Graf, 1976). After inoculation of wheat with *S. nodorum*, yield components were affected, reducing yield by 37 - 43% (Williams and Jones, 1972). Harvest losses between 29 - 31% are reported in wheat in Australia (Bhathal, 2003).

In canary seed, *S. triseti* caused yield reductions of 31% under wet and favorable conditions (May, 2014).

2.4 Fungicide control of *Septoria* spp. and FHB

2.4.1 Fungicide application timing to control *Septoria* spp.

Fungicide application timing is determined by the crop growth stage, and phyllochron (the interval between the emergence of one leaf and the next), as well as the disease latent period and potential disease severity (Paveley et al., 2003). The longer the period of photosynthetically active green leaf tissue, the greater the yield. The optimum fungicide application timing may be similar for

wheat and barley, but the relationship between disease and yield loss may differ. For example, the flag leaf of barley contributes less to yield than the flag leaf of wheat, making an earlier fungicide treatment more effective on barley (Young et al., 2006). In canary seed, there is no information concerning the best fungicide timing application for control of leaf mottle. Successful disease management programs result in high return on investment for growers; fungicides are used to protect crops by controlling pathogens and preventing yield loss. Martens et al. (2014), evaluated the response to fungicide in 45 Canadian wheat cultivars over four years; their study suggested that in 2009, 35 of the cultivars yielded 123% more in fungicide-treated plots than in untreated plots, and in the following year 15 of 45 cultivars yielded 104 % of the untreated plots.

Infection of cereals by *Septoria* spp. that occurs between flag leaf and head emergence is most likely to cause serious yield loss (Eyal, 1961). Complete emergence of the third leaf below the flag leaf (GS 32) and the flag leaf stage (GS 39) of wheat are the two most important fungicide application timing in the UK, which are crucial in the formation of yield (Chang et al., 1974; Pavaley et al., 2012). It has been confirmed that damage to the flag leaf and the ear before the end of grain-filling, about 6 weeks after ear emergence, causes the most damage and greatest yield loss in wheat (Doussinault et al., 1972). Fungicide application to control *S. tritici* on the 3rd leaf below the flag leaf (GS 43 to 51) has been suggested, and a single application at heading has provided good disease control by reducing inoculum on the lower leaves or by protecting the head and flag leaves (Obst and Graf, 1976). In addition, a single application between flag leaf and heading stages when the environmental conditions are conducive for the development of disease results in the largest yield response (Cook, 1977).

Triazoles and strobilurins are the most common fungicides used to control foliar fungal diseases on cereals in North America and Europe (Wegulo et al., 2011). Triazoles are the largest group within the azoles, which have been used to control diseases of wheat since the 1980's (Hollomon et al., 2002). The triazoles (tebuconazole, propiconazole, metconazole and prothioconazole) belong to the DMI (demethylation inhibition) group, which affects the biosynthesis of sterol, required for fungal membranes in the pathogen (FRAC, 2013). Foliar application of triazole fungicides reduced leaf spotting diseases, increased yield and thousand kernel weight of durum wheat in Saskatchewan and Manitoba (May et al., 2014). Application of metconazole to control *S. tritici* improved yield approximately 2 t ha^{-1}, or between 27 and 47% at three locations in the USA (Dooley et al., 2015). Application of propiconazole at head emergence reduced disease severity and increased grain yield of wheat and barley under high levels of leaf spots and rust disease pressure (Entz et al., 1990). Maximum yield increases of 10% in soft white wheat and 3% in hard red spring wheat were recorded when propiconazole was applied at different crop growth stages to control septoria leaf blotch complex in Saskatchewan (Duczek and Jones-Flory, 1994). In addition, applications at flag leaf stage resulted in a 74% yield increase in winter wheat in Sweden (Wiik, 2009). The disease spectrum in wheat controlled by prothioconazole includes septoria leaf spot (*Septoria tritici*) and tan spot (*Drechslera tritici-repentis*), as well as leaf and stripe rust (*Puccinia triticina* and *P. striiformis* f. sp. *tritici*). Beyer et al. (2012) reported that fungicide application in wheat delays development of septoria leaf spot and increases yield up to 3%.

In canary seed, propiconazole has been reported to control leaf mottle caused by *S. triseti* in Saskatchewan. In 1999, control of leaf mottle using propiconazole increased yield up to 22% when disease severity was moderate and 29% when disease severity was high (May, 2002).

Strobilurin fungicides were commonly used to control septoria tritici blotch (STB) in the late 1990s and early 2000s; strobilurins belongs to the QoI (quinone outside inhibitors) group, and affect respiration of the pathogen (FRAC, 2015). In particular, they reduce spore germination and pathogen growth during the latent period (Bartlett et al., 2002). The QoI fungicides control an unusually wide array of fungal diseases, including those caused by water molds, downy mildews, powdery mildews, leaf spotting fungi, and rusts.

Spraying multiple azoles, as a mixture or in sequence, may reduce selection pressure for fungicide insensitivity and yet maintain disease control (Cools and Fraaije, 2013). A fungicide mixture that included cyproconazole, prochloraz and fenpropimorph applied at stem extension and emergence of the flag leaf provided a yield response of 1 t ha $^{-1}$ more than the untreated check in barley by controlling leaf blotch (*Rhynchosporium secalis*) (Young et al., 2006). A combination of triazoles and strobilurins are used to control STB of wheat in Canada. Twinline®, which combines two active ingredients: metconazole and pyraclostrobin, is used to control the septoria disease complex in wheat. Yield improved when combination of tebuconazole, prothioconazole and pyraclostrobin was applied at flag leaf stage (GS65) (Drummond, 2015).

2.4.2 Fungicide control of fusarium head blight

Triazole applications at GS 61 and 65 are recommended to control fusarium head blight (FHB in wheat and late infection by *S. tritici*. Tebuconazole, tebuconazole plus prothioconazole, and pyraclostrobin were very effective in reducing leaf spots from 81.5 to 10.9% of disease severity and FHB from 42.7 to 18.7% disease severity in winter wheat in North Dakota (Ransom and McMullen, 2008). Metconazole is a triazole that has a pronounced effect on fusarium head blight (Bradley et al., 2009). Furthermore, prothioconazole is one of the rare azoles that provide protection against fusarium head blight caused by *Fusarium* spp.

Foliar fungicides are commonly applied to wheat crops at anthesis in the Canadian prairies to control FHB and leaf spot diseases. In addition, application of tebuconazole combined with azoxystrobin at early and mid-anthesis in four wheat cultivars reduced FHB severity; the inoculated treatments included Serio 42%, Genio 80%, Bracco 59% and Duilio 59%, whereas in treatments sprayed with tebuconazole plus azoxystrobin reduction of disease severity reported in sprayed treatments was 23, 32, 15, and 26%, respectively (Haidukowski et al., 2005). Application of tebuconazole before and after FHB inoculation of wheat at late anthesis resulted in reduced FHB, FDK, DON, and glume blotch (*Stagonospora nodorum*) increasing yield by 31 – 80% (Homdork et al., 2000). In addition, application of prothioconazole made at Zadoks growth stage, GS31, GS 39 and GS 65 reduced the FHB incidence by 50, 58, and 83%, and DON content by 27, 49 and 57% compared with untreated check (Edwards and Godley, 2010).

2.5 Summary

In summary, in Saskatchewan leaf mottle caused by *S. triseti* is currently the most common and economically important disease of canary seed. This disease affects lower leaves in the canopy first and results in rapid disease development of the whole plant when weather conditions are favorable on susceptible canary seed genotypes, resulting in yield losses. Studies of the variability in virulence of *S. triseti* are important to detect new sources of resistance and better understand the *S. triseti-P. canariensis* pathosystem. To date, no studies on the virulence of *S. triseti* have been conducted on pathosystem.

Controlling leaf mottle and other potential diseases of canary seed, such as FHB is necessary to reduced yield losses. Fungicide application is a common strategy to control many fungal diseases of cereals. Fungicide application at flag leaf stage have been demonstrated to control leaf diseases on cereals and canary seed. Few studies have been done to determine the best product to control

leaf mottle on canary seed, but studies have not identified the best application timing to reduce yield losses in canary seed. Fusarium head blight is a small grain disease reported in cereals and some grasses. Surveys in recent years have indicated the presence of *Fusarium* spp. in some canary seed commercial fields (Vera et al., 2014; Cholango-Martinez et al., 2015), but no diagnostic, epidemiology or etiological studies of *Fusarium* spp. on canary seed have conducted.

CHAPTER 3:

Variation for virulence of *Septoria triseti* on canary seed (*Phalaris canariensis*) under controlled conditions.

3.1 Introduction

Septoria triseti is the agent causal of leaf mottle on canary seed when environmental and host characteristics (susceptible genotype) are favorable for the development of the disease. *Septoria triseti* is a necrotrophic fungus first reported in Canada in 1988 (Berkenkamp et al., 1989) and has been identified in commercial canary seed crops across Saskatchewan. Environmental conditions are one of the most important factors influencing disease development. For example, in 2014, a year with higher than normal precipitation, leaf mottle disease severity was higher than in 2015, a drier than normal year. In 2014, 50% of the fields surveyed had moderate disease severity (6%-40% of leaf mottle on leaf), whereas in 2015, 87% of the fields had only a trace of disease symptoms (<1%) (Cholango-Martinez et al., 2015; 2016).

Host-pathogen interaction studies provide an understanding of the genetic variability of fungal populations and potential sources of host resistance. Studies of the *Zymoseptoria tritici-wheat* pathosystem have indicated that the *Z. tritici* operates through an isolate-specific mechanism (Kema et al., 1996a, b; Brading et al., 2002; Arraiano and Brown, 2006) and that this pathosystem follows the gene-for-gene model (Flor, 1971).

Control of leaf mottle in canary seed will reduce yield losses. A primary method of control is disease resistant cultivars; therefore, breeding for resistance is required. To date, there is no

understanding of the *S. triseti* - *P. canariensis* pathosystem. Thus, the objective of this project was to determine variation for virulence of *S. triseti* on a selection of germplasm of *P. canariensis* and identify sources of resistance to *S. triseti* in canary seed.

3.2 Material and Methods

3.2.1 Plant Material

This study examined a total of 24 genotypes. Twenty-three genotypes of *P. canariensis*, which included seven cultivars: Cantate, CDC Bastia, CDC Calvi, CDC Maria, CDC Togo, Keet and Elias, and 16 accessions of *P. canariensis* and one *P. brachystachys* accessions obtained from the National Plant Germplasm System USDA (Table 3.1) were used for this study.

3.2.2 *Septoria triseti* isolates

Twenty-seven *S. triseti* isolates were collected from canary seed fields in Saskatchewan: 5 in 2007, 9 in 2013 and 13 in 2014 during field disease surveys (Table 3.2). Ten leaf samples were collected from each field, from which *S. triseti* was isolated by plating the leaf pieces in petri dishes containing wet filter paper. Isolates were placed under light for two to six hours; then cirrhi from individual pycnidia were transferred to PDA (Potato Dextrose Agar) medium. After five days a loop was used to transfer colonies of the pathogen to YMA (Yeast Malt Agar) to increase the number of spores. The spores were incubated in 15% glycerol at 4^{o}C and -15^{o}C for 2 h and 4 h, respectively to reduce thermal shock and then stored at -80^{o}C.

Table 3.1 Identification and origin of 23 genotypes of *Phalaris canariensis* and one genotype of *P. brachystachys* (PI 380967) challenged with 27 isolates of *Septoria triseti* in this study.

ID	Identifier	Origin
1	C05041	Canada
2	Cantate	Netherlands
3	CDC Bastia	Canada
4	CDC Calvi	Canada
5	CDC Maria	Canada
6	CDC Togo	Canada
7	Elias	USA
8	Keet	USA
9	PI 163357	Brazil
10	PI 167261	Turkey
11	PI 170622	Turkey
12	PI 170627	Turkey
13	PI 175811	Turkey
14	PI 175812	Turkey
15	PI 179397	Turkey
16	PI 189547	Mexico
17	PI 203913	Mexico
18	PI 223396	Iran
19	PI 250741	Iran
20	PI 251274	Egypt
21	PI 284180	Morocco
22	PI 284184	Morocco
23	PI 284186	Italy
24	PI 380967	Iran

Table 3.2 Isolates of *Septoria triseti* collected in 2007, 2013 and 2014 from commercial canary seed crops across Saskatchewan evaluated for disease reaction on *Phalaris* spp. genotypes in this study.

ID	Isolate	Sample date	Location
1	07LM1	2007	Indian Head
2	07LM2	2007	Indian Head
3	07LM3	2007	Indian Head
4	07LM4	2007	Indian Head
5	07LM5	2007	Indian Head
6	13LM2	2013	Kyle
7	13LM3	2013	River side No 68
8	13LM4	2013	Cabri
9	13LM5	2013	Cabri
10	13LM6	2013	Netherhill
11	13LM7	2013	N/R
12	13LM8	2013	Richlea
13	13LM9	2013	Richlea
14	13LM10	2013	Coleville
15	14LM1	2014	Wadena
16	14LM2	2014	Madison
17	14LM3	2014	Brock Town
18	14LM4	2014	Eston
19	14LM5	2014	Eston
20	14LM6	2014	Wakaw
21	14LM7	2014	Katepwa
22	14LM8	2014	Indian Head
23	14LM9	2014	Wakaw
24	14LM10	2014	Canora
25	14LM11	2014	Lance Ferry
26	14LM12	2014	Indian Head
27	14LM13	2014	Indian Head

3.2.3 Inoculation of *S. triseti*

Prior to sowing, canary seed were pre-germinated in petri dishes for 7 days. The seeds were wetted and stored in the fridge for 4 days, and then placed in the dark at room temperature for 3 days. When the hypocotyl and the epicotyl appeared, seeds were sowed in root trainers. The trainers each had 32 cells, which were filled with Sunshine Mix no. 4 (Sun Grow Horticulture ® Ltd., Vancouver, BC, Canada) that contained dolomitic limestone, calcium and magnesium. Four cells

were seeded, one seed per cell, with each *Phalaris* spp. genotype, for a total of eight genotypes per trainer and three trainers to accommodate all 24 genotypes.

The isolates were removed from storage at -80 ^{o}C and 100 µl of spore solution was pipetted onto YMA media and cultured in the dark for 3 to 7 days at room temperature. Spores produced on the plates were harvested by pouring a small amount of water onto the plate, and using a loop to rub the culture surface to dislodge the spores. The spores were counted using a hemocytometer and a spore suspension of $1x10^{7}$ spores ml^{-1} was prepared.

Plants were inoculated at the three leaf stage with the spore suspension after mixing with one drop of polyoxyethylene-20-sorbitan monolaurate (Tween 20®), and then sprayed using an atomizer (20 kgf cm^{-2}) over the seedlings. After inoculation, seedlings were put in the humidity chamber for 72 hours under a 16 h photoperiod, 100% relative humidity (RH) and 22^{o}C day/ 18^{o}C night temperatures. Trainers were moved to the growth chamber at 21T^{o}C, 16 h light, 85% RH. The seedlings were fertilized weekly with 20-20-20 (N-P-K) solution.

3.2.4 Disease assessment

Disease assessment was conducted 10 days after inoculation using a 0 – 5 scale (Table 3.3) that has been used to evaluate severity of S. tritici blotch in wheat. Resistance infection type on leaves is characterized by slight necrotic symptoms whereas the susceptible infection type has more pyncnidial development and shows death of tissue. This scale shows clearly the difference between resistant and susceptible which is determinate for the presence or absence of pycnidia formation on the surface of the leave.

Table 3.3 Scale used to evaluate symptoms of *Septoria triseti* on 24 genotypes of canary seed under controlled conditions (McCartney et al., 2002).

Grade	Characteristic
0	Immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms
1	Highly resistance with hypersensitive flecking
2	Resistant with small chlorotic or necrotic lesions, typically **no pycnidial formation**
3	Intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident toward the leaf tips and to a lesser extent elsewhere on the leaf blade, **very light pycnidial formation**
4	Susceptible with moderate pycnidial formation, coalesced necrotic lesions
5	Very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced

3.3 Data analysis

This experiment was arranged in a randomized complete block design. Due to space limitations in the phytotron, the experiment was carried out on separate occasions, each time a different set of isolates was evaluated. The first batch of isolates was collected in 2007 and 2013, and the second batch included isolates collected in 2014. Two replicates were evaluated in each chamber and the experiment was repeated once. Randomization was performed for each replication using MS Excel®.

A host-pathogen interaction was considered susceptible by the presence of pycnidia and resistant if pycnidia were not observed. The scale was used to divide reactions into R (scores ≤ 2.0) and S (> 2.0) groups. An interaction matrix was constructed after grouping the *Phalaris* spp. genotypes with the same interaction phenotypes to summarize the reaction observed between each isolate and each genotype.

3.4 Results

Although there was limited variability among the 27 *S. triseti* isolates, they were categorized into eight groups (pathotypes) based on the host-pathogen interaction response (Table 3.4) . All *P. canariensis* (canary seed) genotypes were susceptible (S) to one isolate, 13LM9, collected at Richlea, SK from CDC Bastia. Isolate 14LM4, collected at Eston, SK caused resistance (R) response on PI 203913. The largest group included 16 isolates from each year of collection (2007, 2013, and 2014) and from numerous locations; Indian Head: 07LM2, 07LM3, 07LM4, 07LM5, 14LM8; Netherhill: 13LM6; N/R: 13LM7; Richlea: 13LM8; Coleville: 13LM10; Wadena: 14LM1; Madison: 14LM2; Brock Town: 14LM3; Easton: 14LM5; Wakaw: 14LM6; Katepwa: 14LM7; and Canora: 14LM10; which had a R response on PI 189547. Isolates from different years and from different sampling sites: Indian Head: 07LM1 and 14LM13; Kyle: 13LM2; River side No68:13LM3; Wakaw: 14LM9, had R response on PI 203913 and PI 189547. Isolate 14LM12 from Indian Head provide a resistance response on PI 203913, PI 189547 and PI 204180. The remaining isolates provided resistance response on four genotypes, but differed from each other: Isolate 13LM5 collected at Cabri caused a resistance response on PI 203913, PI 189547, PI 251274 and Cantate; Isolate 14LM11 collected from Lance Ferry, on PI 203913, PI 189547, PI 163357 and CDC Bastia; and Isolate 13LM4 from Cabri on PI 203913, PI 189547, PI 250741, and CDC Calvi. Among the 27 isolates, provide a R response on all *P. canariensis* genotypes.

One *P. canariensis* line, PI 189547, which originated from Mexico was resistant to 25 of the 27 isolates and line PI 203913, also from Mexico, was resistant to 10 of the 27 isolates. Seven *P. canariensis* genotypes were resistant to only one isolate of *S. triseti*: PI 250741 (Iran) and CDC Calvi (Canada) were resistant to 13LM4, CDC Bastia (Canada) and PI 163357 (Brazil) were resistant to Isolate 14LM11, Cantate (Netherlands) and PI 251274 (Egypt) were resistant to Isolate

13LM5, and PI 284180 (Morocco) was resistant to Isolate 14LM12. Fourteen *P. canariensis* genotypes were susceptible to all *S. triseti* isolates. The *P. brachystachys* line (PI380967) was resistance to all isolates of *S. triseti.*

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Table 3.4 Susceptible (S) and resistant (R) responses caused by 27 *Septoria triseti* isolates collected from canary seed crops in Saskatchewan in 2007, 2013 and 2014, among 23 genotypes of *Phalaris canariensis* (canary seed) and one genotype of *Phalaris brachystachys* (PI380967).

Isolates / *Septoria triseti*	PI380967	PI189547	PI203913	PI250741	CDC Calvi	CDC Bastia	PI163357	PI284180	Cantate	PI251274	C05041	Elias	Keet	CDC Maria	PI167261	PI170622	PI170627	PI175811	PI175812	PI179397	PI223396	PI284184	PI284186	CDC Togo
13LM9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM4	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
07LM2	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
07LM3	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
07LM4	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
07LM5	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM6	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM7	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM8	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM10	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM1	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM2	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM3	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM5	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM6	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM7	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM8	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM10	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
07LM1	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM2	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM3	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM9	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM13	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM5	R	R	R	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM12	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14LM11	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13LM4	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

*Isolate colors indicate different year of origin.

3.5 Discussion

This study was the first to evaluate the disease reaction of *Phalaris canariensis* genotypes challenged with multiple isolates of *Septoria triseti*. To address the research objective of identifying specific interactions between isolates of *Septoria triseti* and *Phalaris canariensis*, we chose scores of >2 and ≤2 on the disease assessment scale to classify the interactions as resistant or susceptible, respectively. This point on the scale was based in the presence or absence of pycnidia on the surface of inoculated leaves. Pycnidia appear after leaf cell collapse in most septoria diseases of other crops, such as cereals (Kema, 1996a). The interaction matrix reported in this study identified specific interactions between *Phalaris* spp. genotypes and *S. triseti* isolates.

Virulence, the ability of the pathogen to cause a S response on a particular host, refers to the interaction between specific genes for virulence and the corresponding resistance genes. Virulence was common among the *S. triseti* isolates on the majority of the *P. canariensis* genotypes. In the gene-for-gene system, only a single incompatible reaction is required to indicate the presence of a gene-for-gene interaction (Flor, 1956). In this pathosystem eight groups of isolates were identified based of their virulence spectra toward 23 *P. canariensis* genotypes. The R and S reactions observed in this study of *P. canariensis* - *S. triseti* may indicate the existence of physiological races. However, further examination of a greater number of isolates would be desirable to conclude the existence of races of *S. triseti*. The largest group included 16 isolates (07LM2, 07LM3, 07LM4, 07LM5, 13LM6, 13LM7, 13LM8, 13LM10, 14LM1, 14LM2, 14LM3, 14LM5, 14LM6, 14LM7, 14LM8, 14LM10), which was the largest pathotype, included isolates collected in three years (2007, 2013 and 2014). These had similar disease reactions on *P. canariensis.* The second largest group was composed of five isolates (07LM1, 13LM2, 13LM3, 14LM9, 14LM13) collected in three years. The smallest groups included just one isolate of each pathotypes (13LM9,

14LM4, 13LM5, 14LM2, 14LM11, 13LM4). Since most of the isolates collected in 2007 showed susceptible response, on 22 of 23 genotypes, compared with isolates collected in 2014 which showed some resistance response, speculate that over years, *S. triseti* may have lost avirulent genes. In the *S. tritici*-wheat pathosystem, Grieger et al. (2005) suggested that the low number of pathotypes observed among the isolates tested was because the pathogen population in western Canada may not be as diverse as that found in other wheat producing regions.

One of the characteristics of the gene-for-gene hypothesis suggested by Person (1959) is the identification of a universal susceptible and a universal virulent. In this pathosystem, isolate 13LM9, collected at Richlea, SK from CDC Bastia, was virulent on the greatest number of Phalaris genotypes; it caused a susceptible reaction on all genotypes of *P. canariensis*. This indicated that this isolate had no avirulence genes that correspond to resistance genes in the *P. canariensis* genotypes examined in this study. On the other hand, Isolates 13LM5, 13LM4 and 14LM11 could be used to screen canary seed germplasm in the future for new sources of resistance. In wheat, the mode of inheritance of resistance to *S. tritici* depends on the aggressiveness of isolates and Bnejdi et al. (2011b) suggested that selection of STB resistant wheat germplasm with less aggressive isolates should be efficient and it will be simple to fix the additive genetic effects; selection with aggressive isolates would be complicated but more stable.

Inoculation with the other 26 isolates resulted in some R reactions among nine canary seed genotypes: PI 189547, PI 203913, PI 250714, CDC Calvi, CDC Bastia, PI 163357, PI 284180, Cantate and PI 251274. Twenty two of 23 genotypes were susceptible to most of the isolates from 2007 (07LM2, 07LM3, 07LM4, 07LM5), whereas the fewest *P. canariensis* genotypes (<18) were susceptible to isolates collected in 2013 and 2014: Isolates 13LM4, 13LM5, and 14 LM11 caused

only R reactions on PI 250741, CDC Calvi, CDC Bastia, PI 163357, Cantate and PI 251274; Isolate 13LM4 on PI 250741 and CDC Calvi; Isolate 13LM5 on PI 251274 and Cantate, and Isolate 14LM11 on CDC Bastia and PI 163357. This indicated that these isolates may have few avirulence genes that correspond to the resistance genes present in these *P. canariensis* genotypes. The response of the isolates on canary seed genotypes indicates differential interactions and therefore the existence of a gene-for-gene system (Flor 1956).

The host genotypes can be classified based on their response to the isolates. Genotypes C05041, Elias, Keet, CDC Maria, PI 167261, PI 175811, PI 175812, PI 179397, PI 223396, PI 284184, PI 284186, and CDC Togo were S to all isolates of *S. triseti*. This indicated they do not carry resistance genes effective at the seedling stage against the isolates examined. Cultivars Elias and Keet have been grown in Saskatchewan since the 1970's. Cultivars CDC Calvi, CDC Bastia, and Cantate are more recent cultivars, but still resistant to only one isolate each, which differed among the cultivars. This may indicate some local adaption in this host-pathogen system, assuming that host and pathogen coevolve.

Lines from Turkey: PI 167261, PI 170622, PI 170627, PI 175811, PI 175812 and PI 179397 were susceptible to all isolates, whereas lines from Mexico, PI 189547 and PI 203913, were resistant to the greatest number of isolates, 25 and 20, respectively. The responses observed for the Mexican lines suggested they may possess at least one and possibly two different resistance genes. The responses suggested that germplasm from Mexico may have similar genetic backgrounds or may share a common gene pool effective against *S. triseti* isolates. The two lines from Mexico may be reliable sources of resistance genes for breeders that can be pyramided to create cultivars with resistance to *S. triseti*.

Plant breeders often search for new resistance genes in wild relatives or primitive cultivars of crops. *Phalaris brachystachys* is reported to be a wild ancestor of canary seed (Oram, 2004). *Phalaris brachystachys* had reactions of up to a score of 1, suggesting that *P. brachystachys* is resistant to *S. triseti.* There are no reports of isolation of *S. triseti* from this species. This study provides comprehensive information of the virulence patterns of *S. triseti* isolates from Saskatchewan and resistance in canary seed genotypes. The results confirmed that the *S. triseti*-*Phalaris* spp. pathosystem can be explained by the gene-for-gene concept describe by Flor (1956). However, it is important to consider an inverse gene-for-gene system since this fungus may produce toxins that may confer a hypersensitive response in the host. The identification of toxins in other species of Septoria such as *Stagonospora nodurum* (Friesen et al., 2007) suggest an inverse gene-for-gene model. More research it is necessary to understand the *Septoria triseti* and canary seed pathosystem.

3.6 Conclusion

The results of this study provide an understanding of the variation in resistance in canary seed germplasm, as well as the virulence of *S. triseti* isolates from Saskatchewan, where most Canadian canary seed is grown. A gene-for-gene interaction was suggested for this pathosystem since specific interactions among pathogen isolates and host genotypes were identified. This study furthers our understanding of the evolution of *S. triseti*.

CHAPTER 4.

Identification of fungal species on canary seed (*Phalaris canariensis*) in Saskatchewan.

4.1 Introduction

Seed of grain crops can be infected by fungal species that may cause yield and quality losses and in severe seed infection may cause storage losses and reduce seed germination. Identification of the species associated with FHB (Fusarium head blight) in canary seed is necessary as a first step to manage the disease. Worldwide many grain crops have been reported to be hosts of *Alternaria* spp. and *Fusarium* spp. Soybean, canola, field pea and some wild grasses were reported to support high levels of *F. graminearum* sporulation (Martinelli et al, 2001; Gilbert et al., 2003b). *Fusarium graminearum* Schwabe is a principal source of FHB on small grain cereal crops and DON content in seed in North America (Cook, 1981). Fusarium head blight not only causes yield losses due to floret sterility, poor seed filling, and reduced germination (Boyacioglu et al., 1992), but also reduces quality caused by mycotoxin contamination (Takana et al., 1988). *Fusarium graminearum* has been prevalent on small grains in eastern Canada and Manitoba for several years (Gilbert and Tekauz, 2000), and since 1994 *F. graminearum* was found in Saskatchewan, mainly on wheat crops at more than trace levels in southeastern SK (Fernandez et al., 2000). *Fusarium graminearum* and Fusarium crown rot have been observed in wheat, barley, rye, oats and triticale (Gordon, 1952; Fernandez et al., 1999; 2000). *Fusarium graminearum* can survive and overwinter in cereals or small grain residues (Sutton, 1982). The most common sources of inoculum are debris from the previous crop season (Gilbert and Fernando, 2004). In the plant residues, perithecia

develop after long periods of wetness at 15 and 25ºC (Dufault et al., 2002a). The ascospores of *F. graminearum* move relatively long distances by air, while conidia are transferred up the plant and from plant-to-plant by rain splash (Hörberg, 2002). Ascospores of *Gibberella zeae* (anamorph *F. graminearum*) have been trapped 60 meter above the ground (Maldonado-Ramirez et al., 2005). When the pathogen and a susceptible host are present and the environmental conditions favorable the disease can be severe. Thus, the objective of this study was to identify the fungal species on canary seed kernels and to evaluate the frequency of *F. graminearum* kernel infection.

4.2 Material and Methods

4.2.1 Seed Material

Seed samples were obtained from 32 unsprayed sub-plots at two locations, Saskatoon and Indian Head. The identification, prevalence and incidence of fungal species on canary seed from commercial fields were determined on 47 samples collected in 2014 and 2015 as a part of field surveys.

4.2.2 Pathogenicity test on seeds

One hundred seeds per sample were surface sterilized in 5% NaClO for 1 min, rinsed three times in sterile water and dried. Seeds were plated on PDA (potato dextrose agar) and placed under a 12 hours light/dark regime at room temperature for five days. Species were identified by shape and size of the macro and micro spores under the compound microscope (magnification 10-100x) using a key for *Fusarium* spp. (Gerlach and Nirenberg, 1982). Colonies were plated separately when fungal identification was inconclusive from the first microscopic observation.

4.2.3 Kochs' postulates for *Fusarium graminearum* on canary seed

Isolate (14FG01) collected from a field at Kindersley, SK (51°14′17.9″ N, 108°49′08.2″ W) was used to prove Koch's postulates. A randomized complete block design experiment of four replications was conducted using cv. Keet, which was seeded three kernels per pot (one replication), and placed in a growth chamber at 22/18°C day/night and a 16 h photoperiod. Canary seed panicles at 50% anthesis were spray inoculated with either a spore suspension (5×10^4 ml^{-1}) of isolate 14FG01 or sterilized water (controls). Plants were harvested 42 days after inoculation (dai), and six panicles per replication were threshed individually. Seeds were hulled, weighed and plated for re-isolation and to determine incidence of *F. graminearum* on canary seed.

4.3 Disease assessment and data analysis

The identity and isolation frequency of each fungus from 100 seeds of each sample was determined. Samples were obtained from fungicide untreated plots at Saskatoon and Indian Head in 2014 and 2015.

The number of seed infected by each *Fusarium* spp. among the 100 plated seed from each canary seed plot was recorded. The seed infection (%) of occurrence of each species was calculated using the following formula:

Seed infection (%) = (number of seeds from which the fungus was isolated/total number of infected seeds) *100

Statistical analysis was done using Proc GLM procedures (SAS Version 9.4, SAS Institute Inc., Cary, NC, USA) to compared differences between inoculated and control treatments.

4.4 Results

4.4.1 Fungal species on canary seed

Of the 3187 seeds examined from the fungicide untreated field plots, 96% were infected with saprophytic and pathogenic fungi. The pathogenic fungi isolated from canary seed were identified as: *F. graminearum* (9.4%), *F. avenaceum* (2.6%), *F. poae* (0.2%), other *Fusarium* spp. (3.9%) and *Cochliobolus* spp. (0.2%); the saprophytic fungi were: *Alternaria* spp. (60.5%), *Cladosporium* spp. (6.5%), *Epicoccum* spp. (5.9%) and another unidentified species (10.9%) (Fig. 4.1).

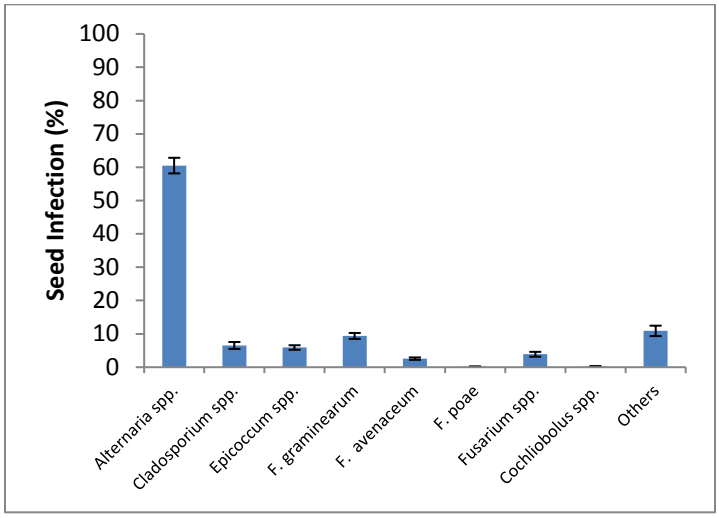

Fig. 4.1 Fungal species present on canary seed. Average of two years: 2014 and 2015 samples. Error bars represent the standard error of the mean (SEM).

There were significant differences between years ($P \leq 0.05$) for *F. graminearum*, *F. avenaceum*, *F. poae* and another *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp., and *Epicoccum* spp., although not for *Cochliobolus* spp. (Table 4.1).

Table 4.1 Incidence (%) of fungal species identified on canary seed kernels from fungicide untreated plots at Saskatoon and Indian Head, 2014 and 2015.

	2014	2015	SEM	*P* value
F. graminearum	12.8	6.0	1.008	**<.0001**
F. avenaceum	1.7	3.4	0.474	**0.0150**
F. poae	0.0	0.3	0.137	**0.0310**
Fusarium spp.	1.6	6.2	1.022	**<.0001**
Cochliobolus spp.	0.0	0.4	0.194	0.1610
Alternaria spp.	50.1	70.9	2.035	**<.0001**
Cladosporium spp.	11.6	1.4	0.809	**<.0001**
Epicoccum spp.	8.5	3.4	0.793	**<.0001**

*Each value is an average of four replicates

Of the pathogenic species, *F. graminearum* was dominant in both 2014 and 2015. For *F. avenaceum* the infection percentage was lower in 2014 (1.7%) than in 2015 (3.4%). *Fusarium poae* had the lowest frequent in canary seed; it was identified only in 2015 (0.3%), as was *Cochliobolus* spp. (0.4%).

4.4.2 *Fusarium* spp. in commercial canary seed crops in Saskatchewan in 2014 and 2015

Four *Fusarium* species: *F. graminearum*, *F. avenaceum*, *F. poae* and *F. equiseti*, were found across Saskatchewan during 2014 and 2015. Prevalence (number of fields infected with fungus from all surveyed field) of *F. graminearum* was higher in 2014 (90%) than in 2015 (58%), as was incidence (proportion of infected seed within a 100 seed sample). *Fusarium poae* was isolated only in 2015, whereas *F. equiseti* was observed only in 2014 (Table 4.2).

Table 4.2 Prevalence of *Fusarium* spp. in commercial fields, and incidence on 100 kernels of each crop in Saskatchewan in 2014 and 2015

	2014		2015	
	Prevalence (%)	Incidence (%)	Prevalence (%)	Incidence (%)
Total *Fusarium* spp.	95	14	88	6
F. graminearum	90	12	58	3
F. avenaceum	48	2	50	1
F. equiseti	14	0.4	-	-
F. poae	-	-	35	1

*Absence of fungus (-)

In 2014, crops in five crop districts were surveyed (Table 4.3), the highest incidence of *F. graminearum* was isolated at Wakaw (73%) (data not shown). In the districts 2B, surrounding Indian Head, and 7A, surrounding Kindersley, three species were identified: *F. graminearum, F. avenaceum* and *F. equiseti*. In Crop Districts 4B and 5B, *F. avenaceum* and *F. equiseti* were isolated. Only *F. graminearum* was isolated from kernels collected from Crop District 8B (Fig. 4.2). In 2015, the highest incidence was at Indian Head (29%). In five of six crop districts, *F. graminearum, F. avenaceum* and *F. poae* were identified. In Crop District 8B, *F. avenaceum* was not present.

Table 4.3 Prevalence (% of crops) of Fusarium spp. in crop districts in Saskatchewan in 2014 (21 crops) and 2015 (26 crops).

Crop District/ Year	Crops	*F. graminearum*	*F. avenaceum*	*F. equiseti*	*F. poae*
2014					
7A	8	75	62	12	0
2B	7	100	57	14	0
4B	2	100	50	0	0
5B	2	100	50	0	0
8B	2	100	0	0	0
2015					
5A	1	100	100	0	100
7A	5	20	20	0	20
2B	11	64	55	0	36
4B	5	40	40	0	20
5B	3	100	100	0	33
8B	1	100	0	0	100

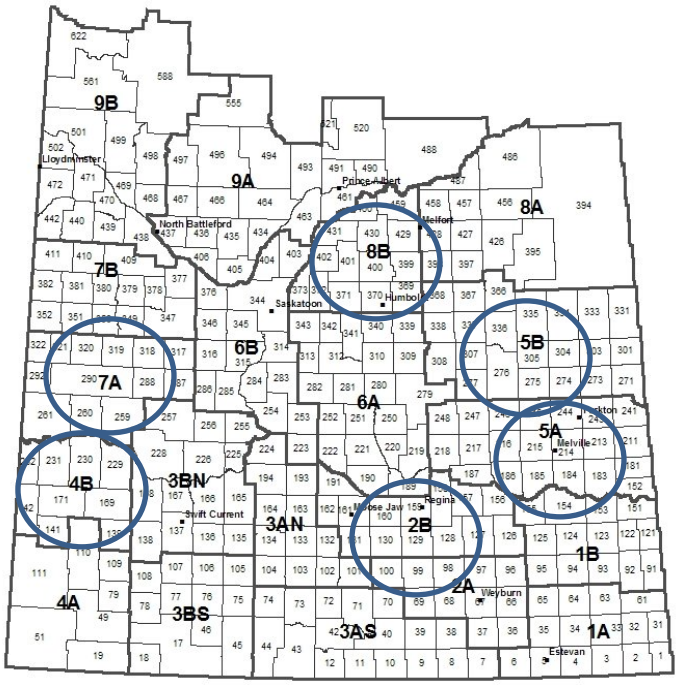

Fig. 4.2 Map of Saskatchewan with crop districts; circles indicate general areas from where canary seed samples were obtained (adapted from: http://agriculture.gov.sk.ca).

4.4.3 *Fusarium graminearum* on canary seed in Saskatchewan

The first visible symptoms, lesions and mycelium, on the panicles appeared 4 dai; at 7 dai some panicles appeared bleached and the peduncle tissues were brown. There were no symptoms on the panicles of the controls. Prematurely ripened seed were common on inoculated panicles, but not on the panicles of the control plants. Prematurely ripened seeds were separated from healthy seeds. Kernels (dehulled seeds) from treated plants were discolored and some were highly shriveled, whereas seeds from the control were plump, of normal color (dark brown), with no visual infection symptoms. Statistical analysis detected differences ($P \leq 0.05$) between treatment and control. There were fewer seeds produced on plants inoculated with *F. graminearum* (175 seeds) compared with the uninfected control (373 seeds), averaged over the six panicles. The 100-kernel weight (g) from the infected plants was (0.53 g) was lower than the control (0.62 g); the incidence of *F. graminearum* infected seed from the treated plants was 28%.

4.5 Discussion

A wide range of saprophytic and pathogenic fungi were detected in canary seed samples. In this study, *Alternaria* spp. were the most frequently isolated fungi on canary seed. *Alternaria* spp. are the most frequent saprophytic fungi reported on cereals. On Danish malt barley *Alternaria* spp. were the most dominant genus of fungi detected (Andersen et al., 1996). Incidence of *Alternaria* spp. up to 86% was reported in wheat, oat and barley (Logrieco et al., 1990). In Norway, 81% of the seed samples of oat, wheat and barley were infected by *A. infectoria* (Kosiak et al., 2004). *Alternaria* spp. (55-66%) were the most common fungi found during 2004 and 2006 on wheat in southeast Saskatchewan (Fernandez et al., 2014). The observation that *Alternaria* spp. were the most common genus of saprophytic fungi observed in canary seed in this study (60.5%) was similar to previous reports on other cereals.

During the 2014 disease survey, orange sporodochia and some pinkish mycelium were observed on the surface of glumes on canary seed; however, the symptomatology of FHB on the seeds was not easily determined in the field. When seeds were plated, *Fusarium* spp. were present on most of the seed. The most common pathogenic specie on canary seed was *F. graminearum* with a prevalence of 95% and 88% in 2014 and 2015. Across Saskatchewan many cereal crops are grown and FHB is common (Clear et al., 2000; Tekauz et al., 2011). The severity of *F. graminearum* reported in durum wheat in Saskatchewan was 32% in 2004 and 59% in 2006 (Fernandez et al., 2014). Del Ponte et al., (2002) reported the presence of ascospores 180 m above the ground and De Luna et al. (2002), observed ascospores movement from point of inoculation to a distance of 60 m. These observations suggest that *F. graminearum* ascospores dispersal into the atmosphere could easily infest the canary seed panicle at anthesis or at any other growth stage after heading.

In cereals, heading stage is reported to be the most susceptible stage that *F. graminearum* can infect the head of the plants (Dill-Macky, 2010). Also, high levels of humidity and temperature combined with the susceptibility of the host may influence the development of FHB. Seed infection (%) of *F. graminearum* on canary seed differed between 2014 and 2015. In 2014, flowering stage in canary seed started in late June when high levels of precipitation (117.3 mm) and temperature of 14.6^{o}C favored the development of FHB on canary seed. In contrast, in 2015, there were dry conditions, temperature was higher (17^{o}C) and precipitation was lower (36 mm) than in 2014. These differences in weather conditions may explain the difference in the prevalence of *F. graminearum* of 95% in 2014 and 88% in 2015. Also, Backhouse and Burgess (2002) suggested that dry weather with high temperatures and moderate to high rainfall can restrict the growth of some *Fusarium* species associated with FHB. Lori et al. (2003), suggested that as rainfall was reduced the incidence of *F. graminearum* decreased, and when RH was above 90% *F.*

graminearum seed infection was high (24.5% and 42%), but when the RH was low (68%) there was no evidence of *F. graminearum*. Although the *F. graminearum* seed infection was present across Saskatchewan, it was most prevalent in the eastern crop district (8B, 5A, 5B, 2B) and less prevalent in the west crop district (7A and 4B) in Saskatchewan province. During 2011, 2012 and 2013 distribution of *F. graminearum* on wheat was most notable in crop district in eastern Saskatchewan (Graefenhan et al., 2014).

Fusarium poae was present in 2015, possibly due to higher temperatures than in the previous year. Kosiak et al. (2004), reported that *F. poae* and *F. culmorum* were favored by warm conditions in Norway during 1997 and 1998. In addition, location seems to influence the prevalence of *F. graminearum* on canary seed in commercial fields in Saskatchewan. *Fusarium graminearum* was prevalent in both years, but in the southwest of the province, less prevalent in 2015 than in 2014. Although the presence or absence of *F. graminearum* could be determined by location, it may also depend on the susceptibility of the host and the amount of inoculum present. However, multiple saprophytic and pathogenic species and their infection processes on canary seed are unclear, thus requiring further research to improve our understanding.

4.6 Conclusion

Fungal species such as *Alternaria* spp., *Cochliobolus* spp., *Cladosporium* spp., *Epicoccum* spp. and *Fusarium* spp. were identified on canary seed in this study. In crop districts where the most canary seed crops were surveyed, 2B and 7A, *F. graminearum*, *F. avenaceum*, *F. equiseti*, and *F. poae* were observed to be associated with fusarium seed infection. This information will facilitate implementation of integrated pest management strategies to control FHB in canary seed and other cereal crops in Saskatchewan, and also to understand better the distribution and new hosts of *F. graminearum* in Saskatchewan.

CHAPTER 5:

Fungicide control of leaf mottle (*Septoria triseti*) and fusarium seed infection on canary seed (*Phalaris canariensis*)

5.1 Introduction

Canary seed is an annual grass that belongs in the Poaceae family, and is used primarily to feed caged birds. Canada is the largest producer of canary seed with an annual seeded area of between 113,000-356,000 ha during the past 10 years. Saskatchewan canary seed growers are responsible for approximately 90% of the Canadian production (Saskatchewan Ministry of Agriculture, 2014). In 2014, seeded area in Saskatchewan was 111,000 ha, an increase from the previous year, which was 85,000 ha. Crop production in 2014 was 124,900 tonnes, approximately 5% lower than that in 2013 (Statistics Canada, 2016). One major reason for reduced canary seed yield is the occurrence of leaf mottle, caused by *Septoria triseti*, which reduces the green leaf area and therefore photosynthesis (Blandino et al., 2009).

Fungicides have been one of the most common strategies used by farmers to control crop diseases to prevent grain yield and quality losses. Leaf mottle of canary seed is controlled by propiconazole in western Canada (Saskatchewan Ministry of Agriculture, 2015). Propiconazole interrupts cell membrane formation of the pathogen, and directly affects biosynthesis of sterol (FRAC code list, 2013). In some other crop types, mixtures of fungicides from two different groups, or rotation of products from two or more groups, such as the strobilurins (Group 11) and triazoles (Group 3) are used to control a broad range of pathogens. One application of prothioconazole + tebuconazole applied between BBCH growth stages 60 and 80 (flowering and ripening) was able to reduce

Zymoseptoria tritici severity by 50% and increase wheat yield by 20% (Rodrigo et al., 2015). In canary seed, 20-40% yield increases were observed after application of fungicides to reduce leaf mottle severity (May et al., 2001). Our hypothesis was that fungicide application at the anthesis stage of canary seed would provide improved leaf mottle and FHB control than would fungicide application at the flag leaf stage. It is essential to identify appropriate fungicide application timing to protect the crop and yield. The objectives of this project were to evaluate the effect of fungicide products, fungicide timings and canary seed genotypes on leaf mottle disease severity, FHB incidence in seed, and yield and quality of the crop.

5.2 Material and Methods

5.2.1 Agronomical conditions

The study was conducted at two locations, Saskatoon at the University of Saskatchewan (lat. 52º07'59.5"N, long. 106º40'12.0"W) and at the Indian Head Research Farm of Agriculture and Agri-Food Canada (lat 50°32'00.2''N, long 103°40'11.6''W), during 2014 and 2015. The canary seed cultivar Keet, which is widely grown by many farmers and the accession PI 251274-3, a genotype believed to be moderately resistant to leaf mottle based on results observed during seedling screening under controlled conditions, where PI 251274-3 had a resistant response to Isolate 07LM02 of *Septoria triseti.*

At Saskatoon in 2014, the field experiment was located near the university at East Sutherland (lat. 52°8'12"N, long. 106°36'14''W). The soil was dark brown (Dark Brown Chernozemic Soils), loam textured with a pH of 6.6; the seeding rate was 250 seeds/m^2 for PI 251274-3 and 500 for Keet. The different seeding rates were based on the percent germination of Keet, which was lower than that of PI 251274-3 as determined previous to seeding. The area of each plot was 16 m^2, 2 x

8 m. The fertilizer applied was 46-0-0, which is urea, at a rate of 33.6 $kgha^{-1}$ of commercial product to supplement the nutrient requirements of 84-95 $kgha^{-1}$ of nitrogen. At Indian Head, the experiment was established at the Indian Head Research Farm of Agriculture and Agri-Food Canada. The plot size was 13 x 35 m, the seeding rate was 250 plants/m^2.

At Saskatoon in 2015, the trial was located on canary seed stubble from a crop grown in 2014. One day before sowing, nitrogen in the form of urea (46-0-0) was applied at a rate of 34 kg ha^{-1} of commercial product was broadcast before seeding and potash at rate of 34 kg ha^{-1} applied at seeding. Two days after sowing herbicides were applied as a mix, glyphosate (Roundup®) 1.6 lha^{-1} and saflufenacil (Kixor®) 0.15 lha^{-1}. At Indian Head 2015, the plots were located on canola stubble and the agronomic conditions were the same as in 2014.

The seeding dates were May 27, 2014 and May 19, 2015 at Indian Head and May 22, 2014 and May 20, 2015 at Saskatoon.

5.2.2 Treatments

The experiments consisted of 14 treatments; seven fungicide treatments applied to two canary seed genotypes: Keet (susceptible) and PI 251274-3 (moderately resistant) (Table 5.1). Three fungicides [prothioconazole + tebuconazole (Prosaro ®), pyraclostrobin + metconazole (Twinline®) and propiconazole (Bumper ®)], were applied at two crop growth stages: flag leaf and or head emergence.

Table 5.1 Fungicide application timing treatments to control leaf mottle and fusarium seed infection on two canary seed genotypes at Saskatoon and Indian Head, Saskatchewan in 2014 and 2015.

Treatment	Genotype	Fungicide	Application timing*	Active ingredient	Rate (ml a. i. /ha)
1	Keet	----------	----------	Unsprayed	-------
2	Keet	Bumper ®	39	Propiconazole	300
3	Keet	Bumper ®	50	Propiconazole	300
4	Keet	Prosaro ®	39	Prothioconazole + tebuconazole	800
5	Keet	Prosaro ®	50	Prothioconazole + tebuconazole	800
6	Keet	Twinline ®	39	Pyraclostrobin + metconazole	500
7	Keet	Twinline® +Bumper ®	39 +50	(pyraclostrobin + metconazole) +propiconazole	500+300
8	PI 251274-3	----------	----------	Unsprayed	-------
9	PI 251274-3	Bumper ®	39	Propiconazole	300
10	PI 251274-3	Bumper ®	50	Propiconazole	300
11	PI 251274-3	Prosaro ®	39	Prothioconazole + tebuconazole	800
12	PI 251274-3	Prosaro ®	50	Prothioconazole + tebuconazole	800
13	PI 251274-3	Twinline ®	39	Pyraclostrobin + metconazole	500
14	PI 251274-3	Twinline® +Bumper ®	39 +50	(pyraclostrobin + metconazole) + propiconazole	500+300

*****BBCH scale (Lancashire et al. 1991): flag leaf (39) and heading (50).

The first fungicide application was made on 15 July 2014 at Saskatoon at the flag leaf stage. Twinline ®, Prosaro ® and Bumper ® were applied at 105, 200 and 125 g a.i. ha^{-1}, respectively. Due to differences in growth stages between the canary seed genotypes, fungicides were applied

at two different two dates for each application timing treatment. Head emergence in Keet was one week later than for PI 251274-3.

5.2.1 Inoculation of *Septoria triseti*

In 2014 at Saskatoon, approximately one month after seeding, when plants were at the five leaf stage, 15 bales (approximately 25 kg per bale) of crop residue from the 2013 trial was spread within the experiment to increase the primary inoculum. In 2015, the experiment was seeded on canary seed stubble, thus canary seed residue was not spread. At Indian Head in 2014, the experiment was seeded on canola stubble but no crop residue was available, and in 2015 the experiment was seeded on canary seed stubble.

5.2.2 Experimental design

The experiments were designed as randomized complete blocks (RCBD) with three factors: fungicide product, application timing and canary seed genotypes with four replicates.

5.2.3 Disease severity assessment on the field

Disease severity ratings were conducted on ten plants per plot on the penultimate and 3rd leaves in each plot. The rating scale used was the Horsfall-Barratt scale (Horsfall and Barratt, 1945) which has 12 grades of disease severity from 0 to 11 (Table 5.2).

Table 5.2 Rating scale used to evaluate leaf mottle severity on canary seed under field conditions (Horsfall and Barratt, 1945).

Grade	Diseased %	Healthy %	Grade formula
0	0	100	1.17
1	0-3	97-100	2.34
2	3-6	94-97	4.68
3	6-12	88-94	9.37
4	12-25	75-88	18.75
5	25-50	50-75	37.50
6	50-75	25-50	62.50
7	75-88	12-25	81.25
8	88-94	6-12	90.63
9	94-97	3-6	95.31
10	97-100	0-3	97.66
11	100	0	98.62

5.2.4 Yield response and seed quality

The harvested grain was weighed after cleaning, to calculate the final yield, then converted to kg ha^{-1}. Thousand kernel weight (TKW), expressed as kg hL^{-1} and oil and protein content (%) were measured from each plot. A subsample of canary seed kernels was dehulled manually and seeds were ground using the RETSCH ZM200 grinder (Retsch GmbH Retsch-Allee 1-5 42781 Haan Germany). The protein extractor LECO FP-528 (3000 Lakeview Avenue, St. Joseph, MI) was used to analyze protein content by using the crude protein-combustion method, which calculates protein based on nitrogen content of the sample. Protein was calculated using the equation: % protein = % N x 5.7 (conversion factor). Oil content was obtained using the fat ANKOM extractor (ANKOM Technology 2052 O'Neil Rd. Macedon, NY).

5.3 Data analysis

Data was analyzed using the SAS mixed model procedure (9.4 SAS Institute Inc. Cary, NC, USA). Prior to analysis, the data from each location was tested for homogeneity using Levene's test.

Heterogeneous variances were modeled with the repeated statement in SAS. Replicate was random, and genotype, fungicide and application timing were fixed factors. Treatments were compared using the Tukey test and significance was declared at $P \leq 0.05$. In addition, two - and three-way interactions were analyzed to identify the effect of factors (fungicide, timing and genotype) in this study and four subset treatments were combined to answer the research objectives; contrast statements were used to compare the unsprayed check with fungicide treatment means.

The four subset treatments presented in Table 5.1 include:

1) Effect of fungicide product, fungicide timing and genotype on canary seed diseases, yield and seed quality: 2 (propiconazole at flag leaf), 3 (propiconazole at heading), 4 (prothioconazole + tebuconazole at flag leaf), 5 (prothioconazole + tebuconazole at heading), 9 (propiconazole at flag leaf), 10 (propiconazole at heading), 11 (prothioconazole + tebuconazole at flag leaf), and 12 (prothioconazole + tebuconazole at heading).

2) Effect of three fungicides applied at flag leaf stage on canary seed diseases, yield and seed quality: 2 (propiconazole), 4 (prothioconazole + tebuconazole), 6 (pyraclostrobin + metconazole), 9 (propiconazole), 11 (prothioconazole + tebuconazole) and 13 (pyraclostrobin + metconazole).

3) Effect of fungicide product applied at heading stage on canary seed diseases, yield and seed quality: 3 (propiconazole), 5 (prothioconazole + tebuconazole), 10 (propiconazole), and 12 (prothioconazole + tebuconazole).

4) Benefit of single and multiple fungicide applications on canary seed diseases, yield and seed quality: 6 (pyraclostrobin + metconazole) at flag leaf, 7 (pyraclostrobin + metconazole at flag leaf

follow by propiconazole at heading stage, 13 (pyraclostrobin + metconazole) at flag leaf and 14 (pyraclostrobin + metconazole at flag leaf follow by propiconazole at heading stage.

5.4 Economic analysis

Economic analysis was calculated using the follow net return fungicide formula: $Rn = Y_iP - (F_c + A_c)$; where Rn was net return from fungicide application ($ ha^{-1}); Y_i was the increase in yield; P was the canary seed price ($ kg^{-1}); Fc was the fungicide cost ($ ha^{-1}) and Ac the fungicide application cost (Wegulo et al., 2011). The estimated cost was based on in-season pricing of propiconazole (Bumper ®) $23.47 ha^{-1}, prothioconazole + tebuconazole (Prosaro ®) $49.54 ha^{-1} and pyraclostrobin + metconazole (Twinline ®) $29.28 ha^{-1}, a canary seed market price of $ 0.51kg^{-1}, and a cost of $17.30 ha^{-1} for application.

5.5 Results

5.5.1 Weather conditions

There was variation in temperature and precipitation between the two years at both locations (Table 5.3). Temperatures were close to long-term normals at each site-year; however, precipitation in 2014 was higher than in 2015. Accumulated precipitation of June and July was higher in 2014 at both locations, Saskatoon (166 mm) and Indian Head (207 mm), compared to 2015, Saskatoon (116 mm) and Indian Head (133 mm) and in July was . Usually canary seed flowering starts in late June and early July.

Table 5.3 Minimum, maximum, and mean monthly temperature (ºC), and precipitation (mm) at Saskatoon and Indian Head, Saskatchewan, from May to August, 2014 and 2015.

	Saskatoon				Indian Head			
Year/month	**Temperature (ºC)**			**Precipitation**	**Temperature (ºC)**			**Precipitation**
	Min.	**Max.**	**Mean**	**(mm)**	**Min.**	**Max.**	**Mean**	**(mm)**
2014								
May	2.5	17.0	10.2	68.6	2.0	18.5	10.2	36 .0
June	9.3	19.6	14.6	**117.3**	9.0	19.8*	14.4	**199.2**
July	11.8	24.4	18.4	48.7	10.7	23.9	17.3	7.8*
August	11.8	24.5	18.0	37.1	11.1	23.6	17.4	142.2
Mean/Total	8.9	21.4	15.3	271.7	8.2	21.5	14.8	385.2
2015								
May	18.3	19.6	11.0	9.6	1.7	18.3	10.0	15.6
June	16.7	17.9	17.6	**33.7**	8.3*	24.2*	16.2	**38.3**
July	18.3	19.6	18.9	82.0	11.6	24.7	18.1	94.6
August	16.7	18.0	17.3	68.5	9.4	24.5	17.0	58.8
Mean/Total	17.5	18.8	16.2	193.8	7.8	22.9	15.3	207.3
Long term avg [a]	9.5	23.0	16.3	49.6	8.6	22.5	15.6	61.0

*The value displayed is based on incomplete data

[a] Long term average 1981-2010

5.5.2 Fungicide treatments response

Means of fourteen treatments are provide in order to have an overall view about the fourteen treatments tested in two years and two locations (Table 5.4).

Table 5.4 Summary of means of fourteen treatments on leaf mottle disease severity, fusarium seed infection, yield, TKW, protein content and oil content of canary seed at Indian Head and Saskatoon in 2014 and 2015.

Treatments	Leaf mottle (%)	Fusarium seed infection (%)	Yield (kg ha^{-1})	TKW (g)	Protein (%)	Oil (%)	Leaf mottle (%)	Fusarium seed infection (%)	Yield (kg ha^{-1})	TKW (g)	Protein (%)	Oil (%)
	Indian Head 2014						***Indian Head 2015***					
1	38.4	11.5	1386	7.5	14.5	6.7	2.1	8.9	1683	7.7	15.6	6.2
2	17.0	11.9	1494	7.6	14.9	7.5	1.7	5.6	1933	7.9	15.8	6.2
3	28.0	7.6	1399	7.6	14.8	7.9	2.5	4.6	1731	7.8	15.6	6.5
4	9.0	11.9	1556	7.6	14.5	7.2	1.5	5.0	1611	7.8	15.6	6.9
5	15.4	7.2	1529	7.6	14.6	7.0	2.0	6.6	1872	8.0	15.3	6.6
6	5.6	5.3	1847	7.6	14.7	7.2	1.6	5.8	1872	7.9	15.4	6.4
7	26.8	10.4	1428	7.5	15.2	7.6	1.5	4.8	1731	7.9	15.4	6.8
8	26.6	13.7	1339	7.5	15.7	6.9	1.4	6.3	1424	7.2	15.7	7.8
9	26.7	11.6	1814	7.8	15.1	7.2	1.5	7.3	1384	7.1	16.1	8.2
10	27.8	13.3	1693	7.7	15.7	7.7	1.5	4.3	1486	7.2	16.2	7.7
11	18.1	4.2	1791	7.9	14.9	8.1	1.5	6.6	1469	7.2	15.7	7.4
12	24.3	9.3	1279	7.8	15.7	7.0	1.6	2.8	1562	7.3	16.1	7.6
13	28.6	7.6	1576	7.9	15.1	7.9	1.2	5.1	1220	7.1	15.6	7.7
14	18.4	12.2	1310	7.8	15.4	7.6	2.5	5.3	1491	7.2	16.1	7.4
	Saskatoon 2014						***Saskatoon 2015***					
1	24.0	12.5	1111	6.8	17.8	6.7	36.8	4.3	958	6.7	16.3	6.8
2	8.0	13.9	1402	7.1	17.6	6.8	14.7	3.6	1347	6.8	15.4	6.8
3	3.3	6.5	1743	7.2	18	7.1	6.1	3.9	1747	7.4	14.5	6.3
4	7.2	10.6	1296	7.0	17.8	7.3	14.4	3.3	1186	6.7	15.9	6.6
5	5.3	2.1	1334	7.2	17.1	7.0	1.9	2.6	1666	7.2	15.6	6.7
6	12.1	13.7	1399	7.1	17.8	7.1	7.6	3.3	1657	6.9	15.7	6.6
7	4.1	9.1	1652	7.3	17.4	7.0	2.2	4.8	1464	7.2	15.1	6.9
8	25.1	13.5	1558	6.1	17.2	7.4	28	4.6	998	6.8	16.1	7.4
9	15.2	15.6	1890	6.5	16.8	8.1	3.2	4.0	1276	6.8	16.2	7.5
10	9.9	12.7	1884	6.4	16.8	7.0	4.0	3.3	1279	7.0	16.1	7.4
11	8.9	14.2	1829	6.5	16.7	8.2	5.2	2.8	1213	6.9	16.3	7.4
12	19.7	2.8	1679	6.3	16.4	7.6	3.2	2.3	1076	7.0	16.1	7.3
13	12.3	9.5	1727	6.5	17.4	7.8	2.9	2.5	1428	7.0	16.0	7.0
14	8.8	12.5	1752	6.6	17.2	8.2	2.3	2.8	1583	7.1	16.0	7.5

5.5.3 Effect of fungicide product, fungicide timing and genotype on canary seed diseases, grain yield and grain quality

Leaf mottle disease severity (%)

In 2014 at Indian Head, fungicide, timing and genotype all had effects on leaf mottle disease severity (Table 5.5). Disease severity was reduced to 24.8% by propiconazole and to 16.7% by prothioconazole + tebuconazole compared with 32.5 in the unsprayed check. Contrast analyses detect differences between unsprayed and sprayed treatments (Fig. 5.1). Genotype PI 251274-3 had higher disease severity (24.2%) than Keet (17.3%) in the unsprayed treatment (Fig. 5.2).

Table 5.5 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	**0.0162**	0.0612	**0.0393**	0.9701	0.5028	0.4249	0.4477
Saskatoon	0.9725	0.8572	0.4698	0.8431	0.8778	0.4798	0.5572
2015							
Indian Head	0.4008	**0.0177**	**0.0052**	0.6887	0.2047	**0.0460**	0.5058
Saskatoon	0.4499	**<.0001**	**<.0001**	0.1414	0.2055	**<.0001**	0.8088

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

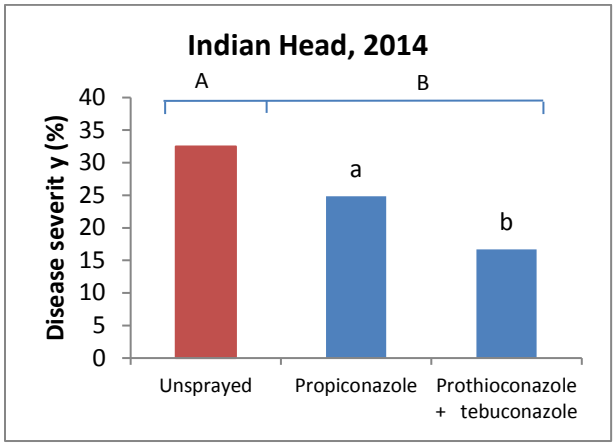

Fig. 5.2 Effect of propiconazole and prothioconazole + tebuconazole on leaf mottle disease severity (%) at Indian Head in 2014. A and B indicate significant differences between unsprayed

and sprayed treatments according to the contrast analysis. Means with the same lower letters are not significant according to the Tukey test.

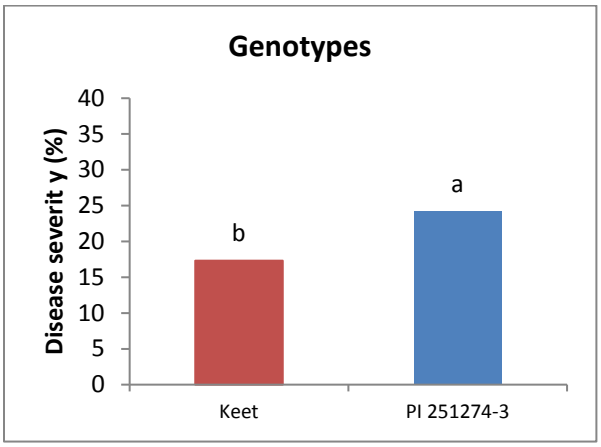

Fig. 5.3 Leaf mottle severity of canary seed genotypes ($P \leq 0.05$) Keet and PI 251274-3 at Indian Head in 2014. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

In 2015 at both locations the interaction of timing and genotype for leaf mottle disease severity was significant (Table 5.5). At Indian Head, fungicide application at the flag leaf stage resulted in similar disease severity of Keet (1.6%) and PI 251274-3 (1.5%), whereas at the heading stage, disease severity of Keet (2.3%) was greater than that PI 251274-3 (1.5%) (Fig. 5.3). At Saskatoon, fungicide application at the heading stage resulted in similar leaf mottle severity for both Keet and PI 251274-3 (3.6%). However, when fungicide was applied at the flag leaf stage, leaf mottle severity was greater for Keet (7.0%) than for PI 251274-3 (4.0%).

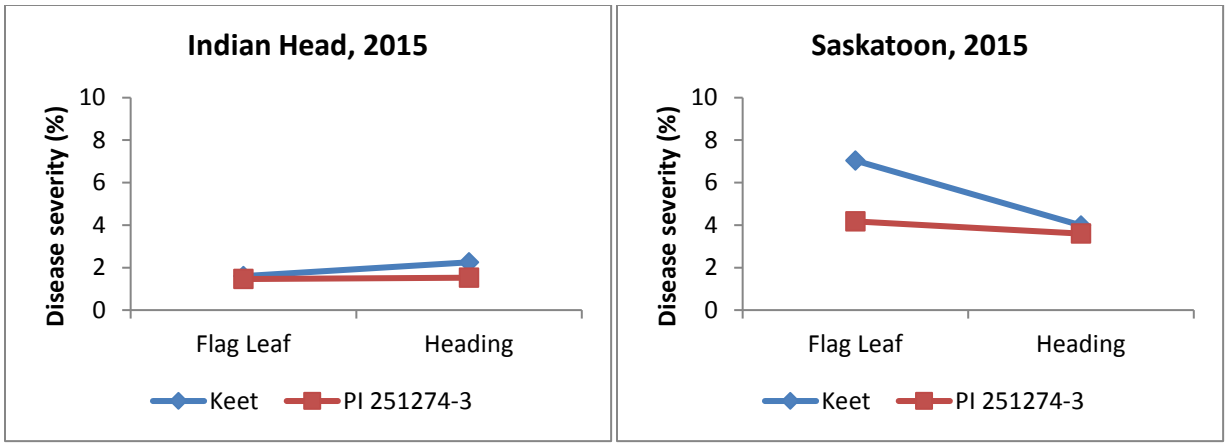

Fig. 5.4 Interaction of variety and fungicide timing effects, on control of leaf mottle of canary seed at Indian Head and Saskatoon in 2015.

Fusarium seed infection (%)

In 2014 at Indian Head and Saskatoon, there was an effect of fungicide application on fusarium seed infection (Table 5.6), which was effectively reduced from the unsprayed check by fungicide treatments. However, the difference between unsprayed check and propiconazole treatment was minimal. The prothioconazole + tebuconazole treatment had lower fusarium seed infection than the propiconazole treatment. Prothioconazole + tebuconazole at Indian Head resulted in 6.4% fusarium seed infection and at Saskatoon 7.4%, while for the propiconazole treatment at Indian Head seed infection averaged 10.8% and at Saskatoon 12.2% (Fig. 5.4). Application timing had an effect on the percentage of seed infected by *F. graminearum* at Saskatoon in 2014 (Table 5.6). Seed infection was higher (13.6%) when fungicide was applied at the flag leaf stage and lower (6.0%) when applied at the heading stage (Fig. 5.5). Contrast analysis indicated that fungicide application at the flag leaf stage was not different from the unsprayed check, however there was a significant different between unsprayed treatment and fungicides sprayed at heading stage. In 2015, there was no effect of fungicide product, application timing or genotype at Saskatoon or Indian Head.

Table 5.6 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on fusarium seed infection (%) at Indian Head and Saskatoon in 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	**0.0053**	0.3166	0.3126	0.1255	0.5983	0.102	0.4592
Saskatoon	**0.0044**	**<.0001**	0.0561	0.1182	0.5649	0.7855	0.2269
2015							
Indian Head	0.8574	0.1444	0.8293	0.6668	0.3733	0.0891	0.4188
Saskatoon	0.0927	0.4568	0.6761	0.7580	0.7748	0.7248	0.5982

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

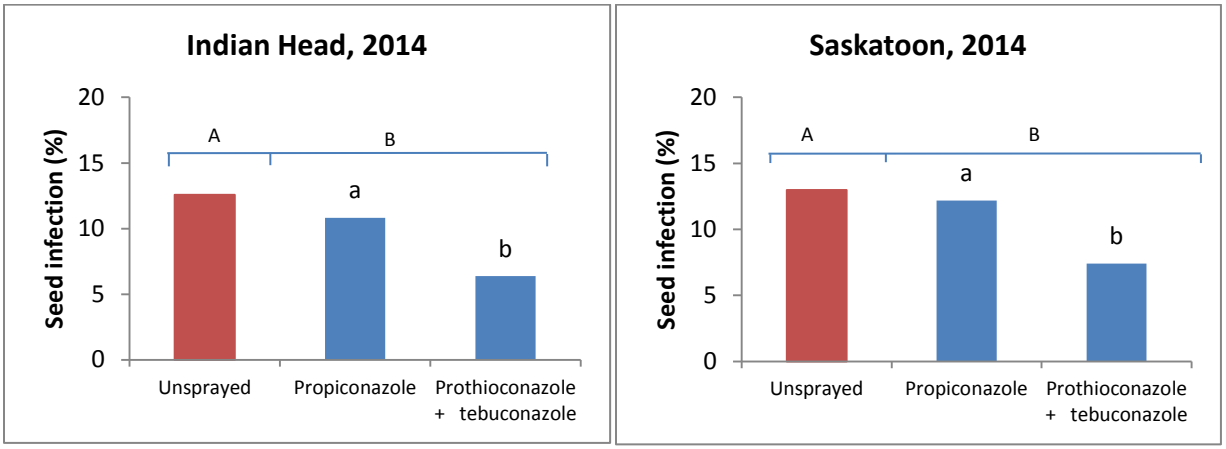

Fig. 5.5 Fusarium seed infection on canary seed after application of propiconazole or prothioconazole + tebuconazole at Indian Head in 2014. A and B show significant differences between unsprayed and sprayed treatments according to the contrast statement. Means with lower case letters indicate differences between sprayed fungicides according to Tukey test ($P \leq 0.05$).

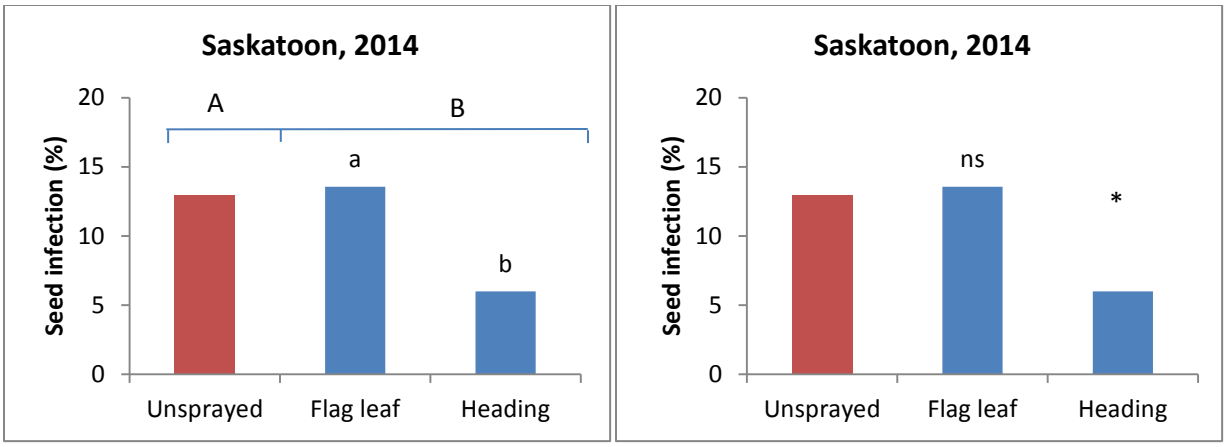

Fig. 5.6 Effect of application timing on fusarium seed infection at Indian Head and Saskatoon in 2014. A and B show significant differences between unsprayed and sprayed treatments according to the contrast statement. Comparison between unsprayed and timing application ns: not significant * significant. Means with lower case letters indicate differences between application timing according to Tukey test ($P \leq 0.05$).

Grain yield (kg ha^{-1})

In two year-sites there was an effect of genotype on yield (Table 5.7). In 2014 at Saskatoon, Keet yield (1444 kg ha^{-1}) was lower than that of PI 251274-3 (1821 kg ha^{-1}), however, at Indian Head in 2015 the opposite occurred, Keet had a higher yield (1787 kg ha^{-1}) than PI 251274-3 (1475 kg ha^{-1}) (Fig. 5.6). In 2015 at Saskatoon, the interaction of fungicide application timing and genotype was statistically significant (Table 5.7). When fungicide was applied at the heading stage, Keet had a higher yield (1706 kg ha^{-1}) than PI 251274-3 (1177 kg ha^{-1}). Yield for both genotypes was similar when fungicide was sprayed at the flag leaf stage: Keet (1267 kg ha^{-1}) and PI 251274-3 (1245 kg ha^{-1}) (Fig. 5.7).

Table 5.7 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on yield (kg ha^{-1}) at Indian Head and Saskatoon in 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	0.5481	0.0723	0.1494	0.4280	0.1298	0.2158	0.2636
Saskatoon	0.1202	0.5665	**0.0013**	0.1424	0.3528	0.5834	0.4020
2015							
Indian Head	0.9652	0.5573	**0.0082**	0.3014	0.4312	0.7536	0.2822
Saskatoon	0.3061	0.1379	**0.0330**	0.9019	0.9598	**0.0477**	0.6536

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

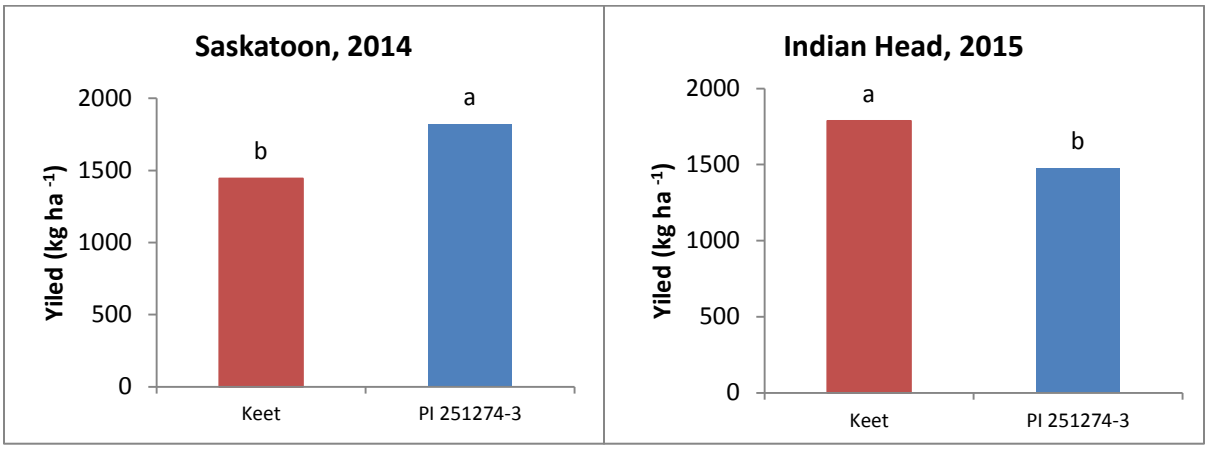

Fig. 5.7 Yield of two canary seed genotypes Keet and PI 251274-3 at Saskatoon 2014 and Indian Head in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

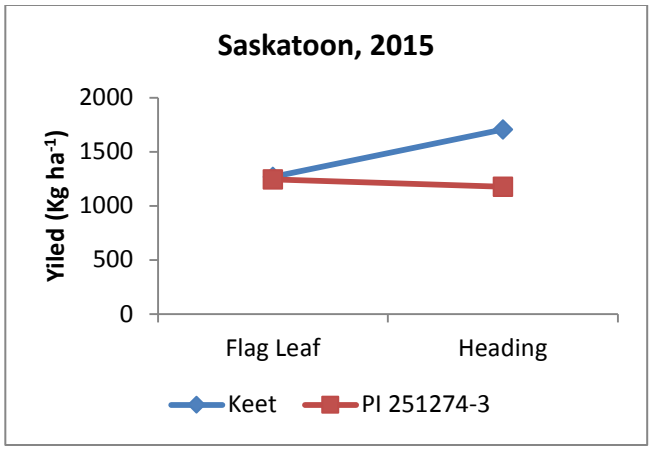

Fig. 5.8 Interaction of genotypes and timing on yield of canary seed at Saskatoon in 2015.

Grain quality traits

Thousand kernel weight (g)

In three site-years (Indian Head and Saskatoon in 2014, and Indian Head in 2015) genotype had an effect on TKW (Table 5.8). However, the effect was not consistent. At Indian Head in 2014, Keet (7.6 g) had a lower TKW (g) than PI 251274-3 (7.8 g), whereas at Saskatoon in 2014 and at Indian Head in 2015, Keet (7.1 g and 7.8 g) had a higher TKW than PI 251274-3 (6.4 g and 7.2 g) (Fig. 5.8).

Table 5.8 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on grain quality traits on canary seed at Indian Head and Saskatoon in 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	0.3978	0.3978	**0.0018**	0.7151	0.5441	0.2796	0.9030
Saskatoon	0.8044	0.9342	**<.0001**	0.9342	0.8044	0.1272	0.4602
2015							
Indian Head	0.2865	0.2865	**<.0001**	0.1407	0.5189	0.5189	0.1407
Saskatoon	0.4476	**0.0021**	0.3029	0.4476	0.3706	**0.0396**	0.9446

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

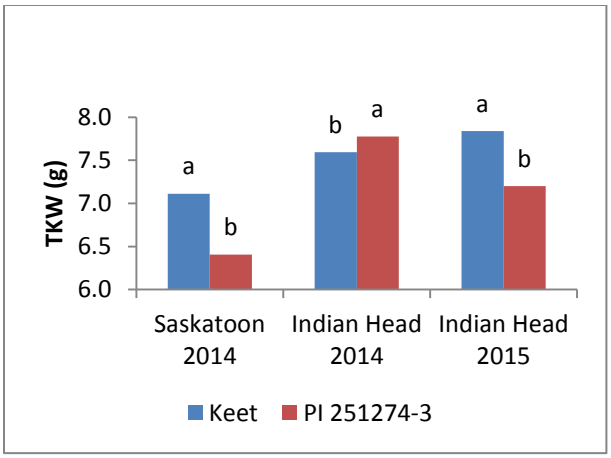

Fig. 5.9 Thousand Kernel Weight (g) of two canary seed genotypes (PI 251274-3 and Keet) at Saskatoon and Indian Head in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

In 2015 at Saskatoon, the fungicide application timing and genotype interaction was significant (Table 5.8). Thousand kernel weight of PI 251274-3 was similar when fungicide was applied at either growth stage, however for Keet TKW was higher when fungicide was applied at heading (7.3 g) than at the flag leaf stage (6.8 g) (Fig. 5.9).

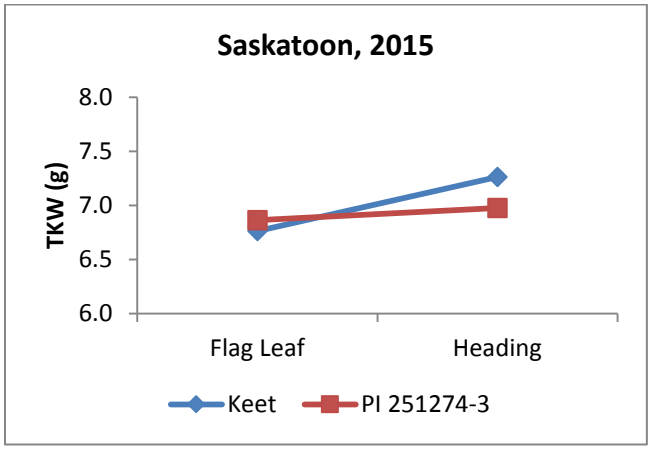

Fig. 5.10 Interaction of timing and genotype on thousand kernel weight on canary seed at Saskatoon in 2015.

Protein content (%)

Genotype had an effect on protein content at both sites and in both years (Table 5.9). Protein content of the canary seed was greater for PI 251274-3 than for Keet at Indian Head in both years (2014 and 2015) and at Saskatoon in 2015 only (Fig. 5.10). The opposite occurred at Saskatoon in 2014, where protein content of Keet was greater than that of PI 251274-3.

Table 5.9 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on grain quality traits on canary seed at Indian Head and Saskatoon in 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	0.2443	0.0680	**0.0014**	0.5438	0.6419	0.0590	0.9714
Saskatoon	0.5191	0.8609	**0.0065**	0.2092	0.7729	0.6241	0.9561
2015							
Indian Head	0.1756	0.9448	**0.0170**	0.8355	0.9448	0.2748	0.5810
Saskatoon	0.0677	0.1067	**0.0011**	0.5537	0.0955	0.3178	0.3768

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

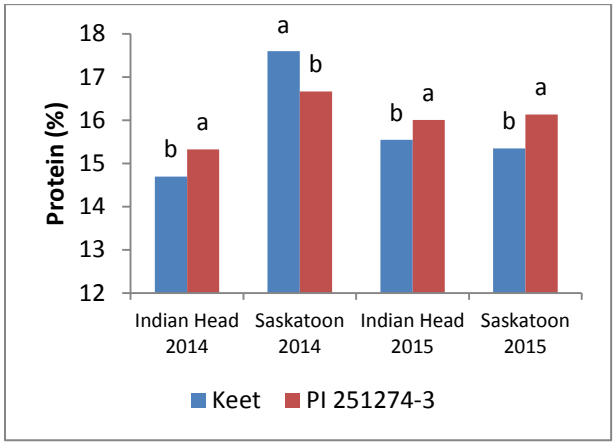

Fig. 5.11 Protein content of canary seed genotypes, Keet and PI 251274-3 at Saskatoon and Indian Head in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Oil content (%)

The interaction of fungicide and timing had an effect on oil content of canary seed in 2014, at Indian Head (Table 5.10), prothioconazole and prothioconazole + tebuconazole at the flag leaf stage had higher oil content than at the heading stage. At Saskatoon in 2014, the interaction of timing and genotype had an effect on oil content, and at Indian Head in 2015 there was an effect of fungicide, timing and genotype on oil content. Finally, at Saskatoon in 2015, only genotype had an effect on oil content. At Indian Head in 2014, the interaction between fungicide and timing was because fungicide application at flag leaf stage resulted in higher oil content than fungicide application at heading stage for both fungicide products, but the difference in oil content between fungicide application stages was greater for prothioconazole + tebuconazole than for propiconazole (Fig. 5.11).

Table 5.10 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole), timing (leaf and heading stages) and genotype (Keet and PI 251274-3) on grain quality traits on canary seed at Indian Head and Saskatoon in 2014 and 2015.

Year/Factor	Fungicide (F)	Timing (T)	Genotype (G)	FxT	FxG	TxG	FxTxG
2014							
Indian Head	0.1926	0.6582	0.6163	**0.0146**	0.1148	0.3069	0.2808
Saskatoon	0.1445	**0.0412**	**0.0016**	0.8964	0.6965	**0.0360**	0.2243
2015							
Indian Head	0.8459	0.6696	**<.0001**	0.8459	**0.0257**	0.6696	**0.0494**
Saskatoon	0.9735	0.5294	**0.0004**	0.3401	0.6189	0.8160	0.4874

Degree of freedom: 1 for F, 1 for T, 1 for G and 1 for (FxT, FxG, TxG and FxTxG). Significant differences were indicated by ($P \leq 0.05$).

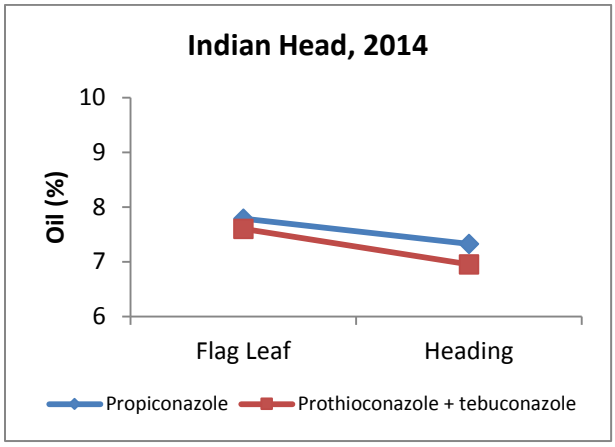

Fig. 5.12 Interaction of fungicide and timing on oil content of canary seed genotypes Keet and PI 251274-3 at Indian Head in 2014.

At Saskatoon, the oil content of Keet was similar with both fungicide application timings, flag leaf (7.0%) and heading (7.1%), whereas, the oil content of PI 251274-3 was higher when fungicide applications where made at the flag leaf stage (8.2%) versus the heading stage (7.3%) (Fig. 5.12).

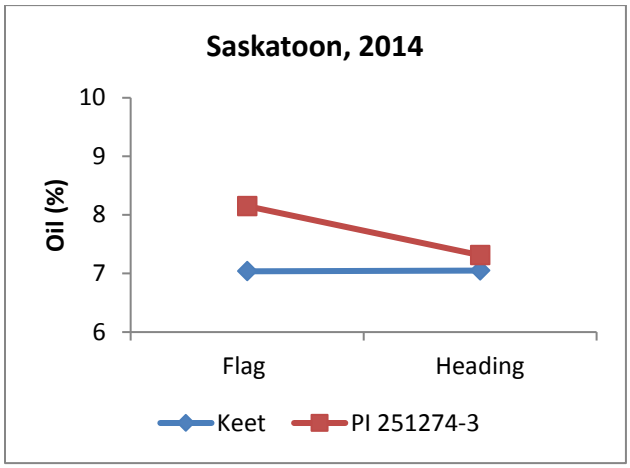

Fig. 5.13 Interaction of timing and genotype on oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon in 2014.

In 2015 at Indian Head, the interaction of fungicide, timing and genotype was statistically significant (Table 5.10). Keet had a lower oil content than PI 251274-3. Application of propiconazole on Keet at flag leaf stage (6.2%) resulted in a lower oil content than the application at heading (6.5%). Prothioconazole + tebuconazole applied to Keet at the flag leaf stage had higher oil content (6.9%) than the application at the heading stage (6.6%). The highest oil content (8.2%) was for PI 251274-3 sprayed with propiconazole at the flag leaf stage (Fig. 5.13).

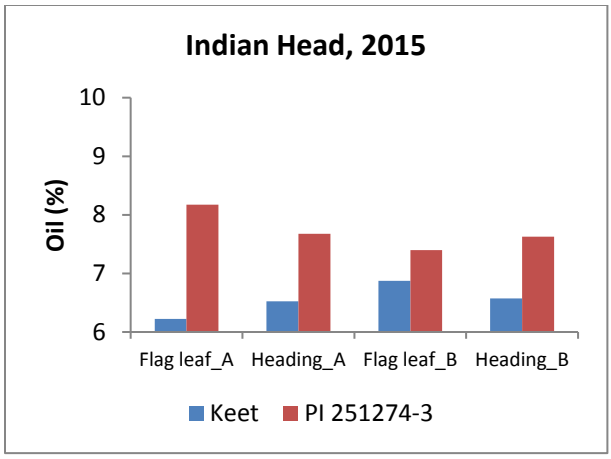

Fig. 5.14 Interaction of fungicide product, fungicide application timing, and genotype, A: propiconazole and B: prothioconazole + tebuconazole, on oil content on canary seed at Indian Head in 2015.

At Saskatoon in 2015, genotype was the only factor that had an effect on oil content (Table 5.10). Keet had a lower oil content (6.6%) than PI 251274-3 (7.4%) (Fig. 5.14).

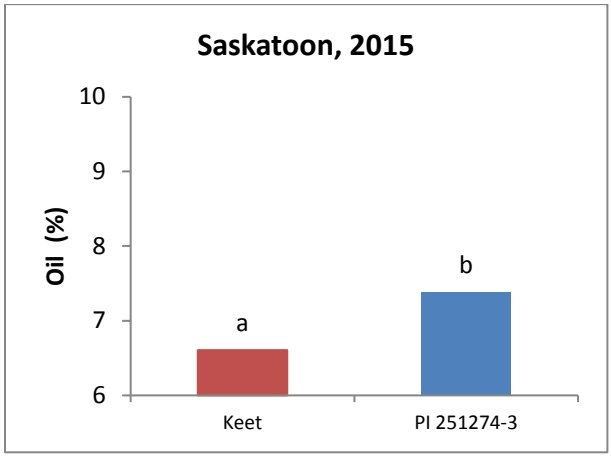

Fig. 5.15 Oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

5.5.4 Effect of three fungicides applied at the flag leaf stage on canary seed diseases, grain yield and grain quality

Leaf mottle disease severity (%)

In 2014 at Indian Head, prothioconazole + tebuconazole and pyraclostrobin + metconazole applications at the flag leaf stage (Treatments 4 and 11 and 6 and 13, respectively) reduced leaf mottle severity compared to the unsprayed check. Leaf mottle severity was not different from the unsprayed check for the propiconazole treatment (Table 5.11). At Saskatoon in 2014, leaf mottle was reduced to 8% after application of prothioconazole + tebuconazole, compared with 24.6% for the unsprayed check. The prothioconazole + tebuconazole and pyraclostrobin + metconazole treatments did not differ statistically from the unsprayed check. In 2015 at Indian Head, leaf mottle disease severity was extremely low and there were no fungicide treatment differences. At Saskatoon in 2015, leaf mottle severity was reduced by all fungicides applied: propiconazole (9%), prothioconazole + tebuconazole (9.8%) and pyraclostrobin + metconazole (5.3%) compared with the unsprayed check (32.4%).

Fusarium seed infection (%)

Fungicide products reduced fusarium seed infection in two year-sites (Table 5.11). Two fungicide products reduced fusarium seed infection ($P \leq 0.05$) at Indian Head in 2014 (Table 5.6), prothioconazole + tebuconazole (4.5%) and pyraclostrobin + metconazole (6.5%) compared with the unsprayed check (12.6%) or propiconazole (11.2%). At other site-years there was no effect of fungicide on fusarium seed infection.

Grain yield (kg ha^{-1})

Yield of canary seed was increased by fungicide application at one site-year Saskatoon only in 2015 (Table 5.11), but only after application of pyraclostrobin + metconazole (1542 kg ha^{-1}) compared with the unsprayed check treatment (978 kg ha^{-1}).

Grain quality traits

Thousand kernel weight (g), protein content (%) and oil content (%)

There were no effects in thousand kernel weight, or protein or oil content of canary seed for any of the fungicide products in any site-year (Table 5.11).

Table 5.11 Effect of three fungicides: propiconazole, prothioconazole + tebuconazole and pyraclostrobin + metconazole, at flag leaf stage on canary seed at Indian Head and Saskatoon in 2014 and 2015

Year/ Response	Location	Unsprayed	Propiconazole	Prothioconazole + tebuconazole	Pyraclostrobin + metconazole	SEM	*P* value
Leaf mottle disease severity (%)							
2014	Indian Head	32.5 a	21.8 ab	13.5 b	17.1 b	4.07	**0.0158**
	Saskatoon	24.6 a	11.6 ab	8.0 b	12.2 ab	8.31	**0.0314**
2015	Indian Head	1.7	1.6	1.5	1.4	0.12	0.2366
	Saskatoon	32.4 a	9.0 b	9.8 b	5.3 b	2.07	**<0.0010**
Fusarium seed infection (%)							
2014	Indian Head	12.6 a	11.2 a	4.5 b	6.5 b	1.31	**0.0004**
	Saskatoon	13.0	14.7	12.4	11.6	1.71	0.6152
2015	Indian Head	7.6	6.4	5.8	5.5	0.98	0.4186
	Saskatoon	4.4	3.8	3.0	2.9	1.34	0.0549
Grain yield (kg ha^{-1})							
2014	Indian Head	1362	1654	1673	1711	115	0.0928
	Saskatoon	1334	1646	1562	1563	137	0.3837
2015	Indian Head	1553	1658	1540	1546	145	0.9048
	Saskatoon	978 b	1311 ab	1200 ab	1542 a	131	**0.0098**
Thousand kernel weight (g)							
2014	Indian Head	7.5	7.7	7.7	7.7	0.07	0.0926
	Saskatoon	6.5	6.8	6.6	6.8	0.14	0.3717
2015	Indian Head	7.4	7.5	7.5	7.5	0.14	0.9702
	Saskatoon	6.7	6.8	6.8	6.9	0.10	0.3203
Protein (%)							
2014	Indian Head	15.1	15	14.7	14.9	0.17	0.3742
	Saskatoon	17.5	17.2	17.2	17.6	0.44	0.4939
2015	Indian Head	15.7	15.9	15.6	15.5	0.28	0.4516
	Saskatoon	16.2	15.8	16.1	15.9	0.32	0.5699
Oil (%)							
2014	Indian Head	6.8	7.3	7.6	7.6	0.27	0.1558
	Saskatoon	7.0	7.4	7.8	7.5	0.28	0.3620
2015	Indian Head	7.0	7.2	7.1	7.0	0.33	0.9587
	Saskatoon	7.1	7.2	7.0	6.8	0.28	0.7247

Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$). SEM=Standard error of the mean.

5.5.5 Effect of fungicide product applied at heading stage on canary seed diseases, grain yield and grain quality.

Leaf mottle disease severity (%)

There was an effect of treatments in two of four site years (Table 5.12). At Indian Head in 2015, genotype had an effect on leaf mottle severity. Keet had a higher disease severity (2.3%) than PI 251274-3 (1.5%) (Fig. 5.15).

Table 5.9 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	FxG
2014	Indian Head	0.1787	0.4534	0.4310
	Saskatoon	0.3687	0.1266	0.5480
2015	Indian Head	0.5287	**0.0238**	0.3300
	Saskatoon	0.0719	0.7557	0.2019

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

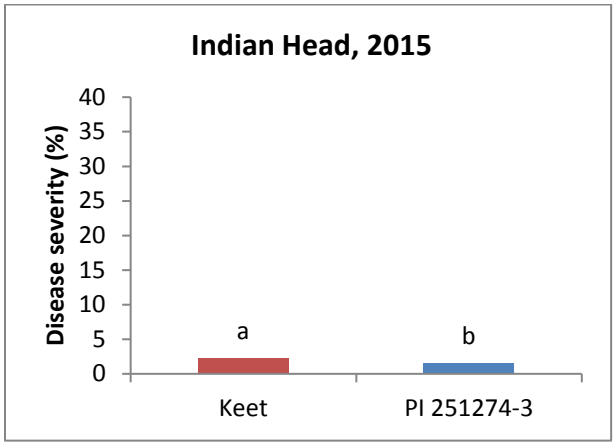

Fig. 5.16 Leaf mottle severity of canary seed genotypes Keet and PI 251274-3 at Indian Head in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Fusarium seed infection (%)

In one site-year (Saskatoon in 2014), there was an effect of fungicide ($P \leq 0.05$) on the incidence of fusarium infected seed (Table 5.13). Prothioconazole + tebuconazole had lower fusarium seed infection (2.4%) than propiconazole (9.6%) (Fig 5.16). Contrast analysis between unsprayed and sprayed treatments was significant ($P \leq 0.05$).

Table 5.10 Probability of *F* values for the analysis of variance for fungicide and genotype on fusarium seed infection (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	F x G
2014	Indian Head	0.2530	0.0545	0.3265
	Saskatoon	**0.0013**	0.0552	0.1135
2015	Indian Head	0.8609	0.1901	0.2506
	Saskatoon	0.2826	0.6772	0.8955

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

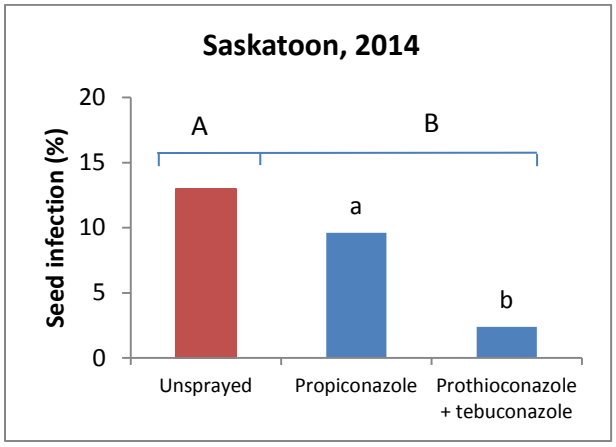

Fig. 5.17 Fusarium seed infection on canary seed after application of propiconazole and prothioconazole + tebuconazole at Saskatoon, 2014. Means with lower or upper case letters indicate differences between treatments according to Tukey test ($P \leq 0.05$).

Grain yield (kg ha^{-1})

There was an effect of treatments in three of four site-years (Table 5.14). At Saskatoon in 2014, there was a yield difference between fungicide treatments. In 2015, at Saskatoon genotype effect was significant and yield of Keet was always higher than PI 251275-3 (Fig. 5.17).

Table 5.11 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on yield (kg ha^{-1}) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	FxG
2014	Indian Head	0.3641	0.8863	0.1001
	Saskatoon	0.0982	0.1789	0.5563
2015	Indian Head	0.4046	0.0520	0.8012
	Saskatoon	0.4498	**0.0162**	0.7411

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

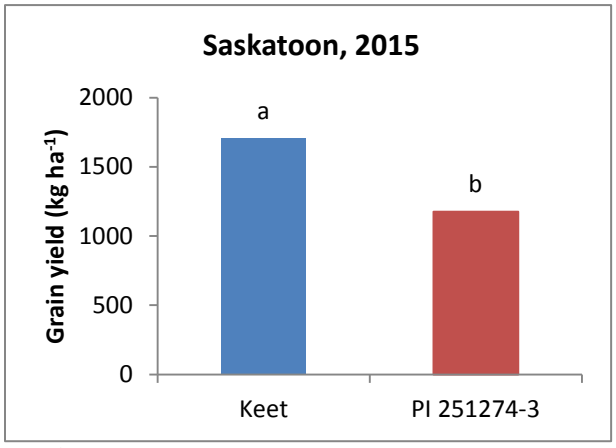

Fig. 5.18 Grain Yield (kg ha^{-1}) of two genotypes of canary seed after application of propiconazole or prothioconazole + tebuconazole at Indian Head. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Grain quality traits

Thousand kernel weight (g)

In three site-years genotype had an effect on TKW at $P \leq 0.05$ (Table 5.15). Except for Indian Head in 2014, thousand kernel weight for Keet was higher than that of PI 251274-3 (Fig. 5.18).

Table 5.12 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on TKW (g) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	FxG
2014	Indian Head	0.6843	0.0651	0.6843
	Saskatoon	0.8321	**<.0001**	0.5291
2015	Indian Head	0.0963	**<.0001**	0.5514
	Saskatoon	0.2713	**0.0367**	0.4749

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

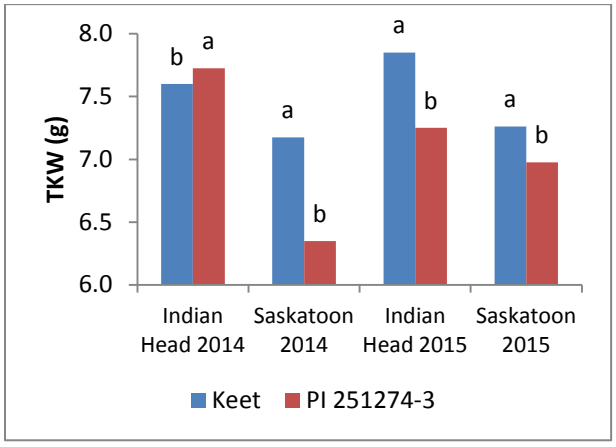

Fig. 5.19 Thousand Kernel Weight (g) of two canary seed genotypes (Keet and PI 251274-3) to control leaf mottle at Saskatoon and Indian Head in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Protein content (%)

In two site-years genotype had an effect on protein content ($P \leq 0.05$) (Table 5.16). In 2014, at Saskatoon, Keet had a higher protein content (17.5%) than PI 251274-3 (16.6%), whereas at Indian Head in 2015, Keet had lower protein content (15.4%) than PI 251274-3 (16.1%) (Fig. 19). At Saskatoon in 2014, protein content after application of propiconazole (17.4%) was higher than with the application of prothioconazole + tebuconazole (16.8%) (Fig. 20). However, a contrast between spray applications did not detected an effect on protein compared with unsprayed treatments. Protein was lower for Keet after application of prothioconazole + tebuconazole than with propiconazole, whereas for PI 251274-3 there was no difference in protein content after application of either propiconazole or prothioconazole + tebuconazole (Fig. 20).

Table 5.13 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on protein (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	FxG
2014	Indian Head	0.7174	**0.0054**	0.7857
	Saskatoon	**0.0466**	**0.0077**	0.4282
2015	Indian Head	0.4736	**0.0446**	0.7013
	Saskatoon	0.1110	**0.0083**	0.0972

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

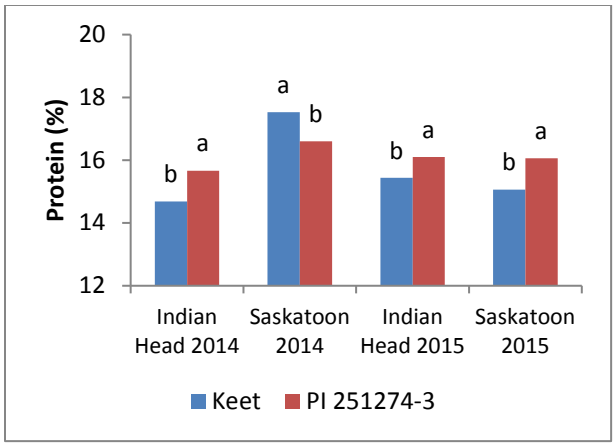

Fig. 5.20 Protein content of canary seed genotypes, Keet and PI 251274-3 after application of propiconazole and prothioconazole + tebuconazole at Saskatoon and Indian Head in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

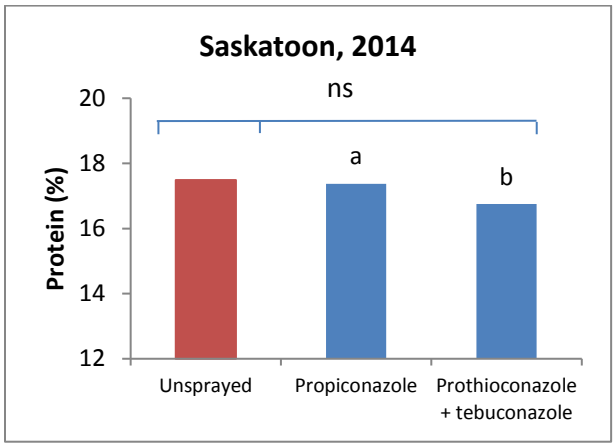

Fig. 5.21 Protein content of canary seed genotypes after application of propiconazole or prothioconazole + metconazole at Saskatoon 2014. Contrast statement between unsprayed and sprayed treatments, were non significance (ns). Means with lower case letters indicate differences between fungicides according to Tukey test ($P \leq 0.05$).

Oil content (%)

At three site-years of four there was an effect of fungicide treatment on oil content of canary seed (Table 5.17). In 2014 at Indian Head, fungicide treatment increased oil content over the unsprayed treatment (Fig 5.21). Between fungicide treatments, propiconazole had a higher oil content than

prothioconazole + tebuconazole. In 2015 at both locations, genotype had an effect on oil content (Table 5.16); Keet had lower oil content than PI 251274-3 (Fig. 5.22).

Table 5.14 Probability of *F* values for the analysis of variance for fungicide (propiconazole and prothioconazole + tebuconazole) and genotype (Keet and PI 251274-3) on oil (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Fungicide (F)	Genotype (G)	FxG
2014	Indian Head	**0.0014**	0.5555	0.5555
	Saskatoon	0.2817	0.2817	0.2059
2015	Indian Head	1.0000	**<.0001**	0.6193
	Saskatoon	0.4933	**0.0083**	0.3825

Degree of freedom: 1 for F, 1 for G and 1 for FxG. Significant differences were indicated by ($P \leq 0.05$).

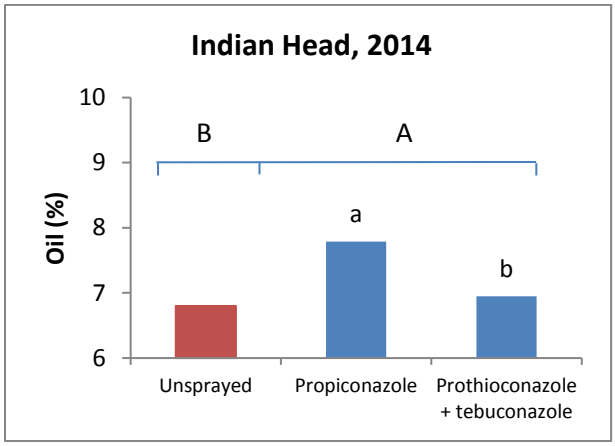

Fig. 5.22 Oil content after application of two fungicides on canary seed at Indian Head in 2014. A and B show significant differences between unsprayed and sprayed treatments according to the contrast statement. Means with lower case letters indicate differences between fungicides according to Tukey test ($P \leq 0.05$).

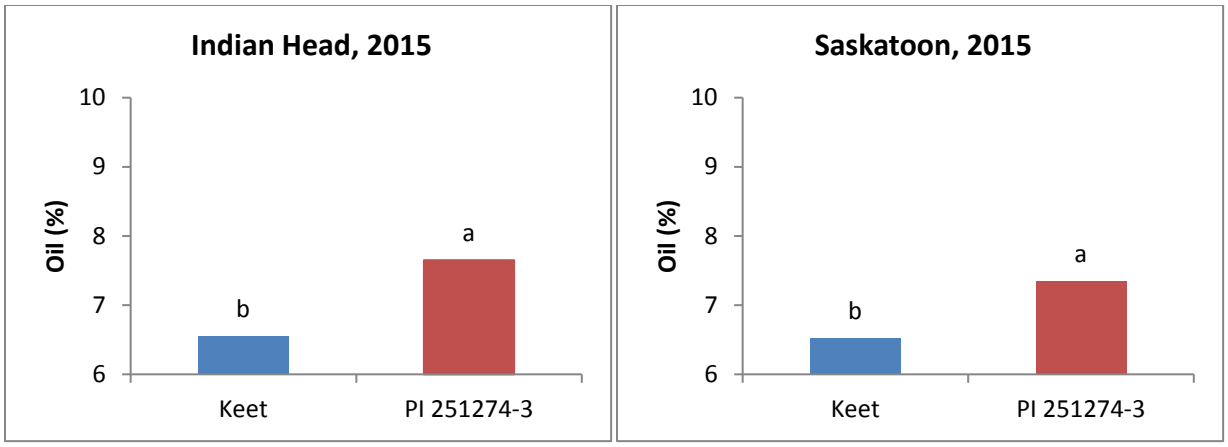

Fig. 5.23 Oil content of canary seed genotypes Keet and PI 251274-3 at Saskatoon and Indian Head in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

5.5.6 Benefit of single and multiple fungicide applications on canary seed diseases, grain yield and grain quality

Leaf mottle disease severity (%)

In Indian Head in 2014, the interaction of frequency of fungicide application (at flag leaf stage or at flag leaf and heading stages) and genotype was significant (Table 5.18). In 2014 at Indian Head, one application of pyraclostrobin + metconazole at the flag leaf stage controlled disease severity more effectively on Keet (5.6%) than in PI 251274-3 (28.6%), whereas two applications of fungicides: pyraclostrobin + metconazole at leaf stage follow by propiconazole at heading stage, on PI 251274-3 (18.4%) controlled leaf mottle better than on Keet (26.8%) (Fig. 5.23). In Saskatoon 2015 frequency of fungicide application was significant different. Fungicides application reduced disease severity compared the unsprayed treatment 32.4% disease severity with sprayed treatments 3.8% (average of fungicide treatments) (Fig. 24). One fungicide application (5.3%) had higher disease severity than two fungicides applications (2.2%).

Table 5.15 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on leaf mottle (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	0.1567	0.0731	**0.0017**
	Saskatoon	0.1453	0.5116	0.5461
2015	Indian Head	0.2404	0.4579	0.1618
	Saskatoon	**0.0213**	0.0660	0.0568

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

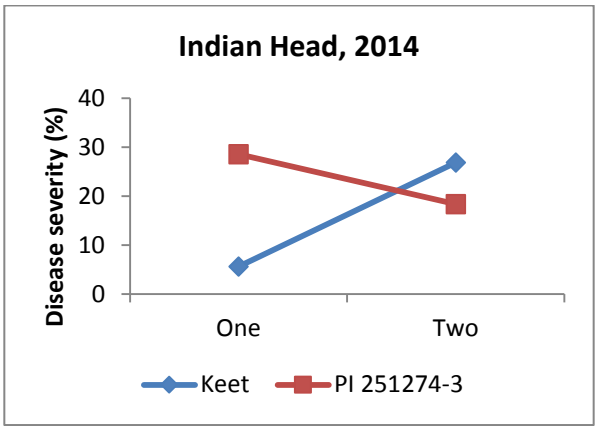

Fig. 5.24 Interaction of two factors, fungicide and genotype, to control leaf mottle on canary seed at Indian Head ($P \leq 0.05$).

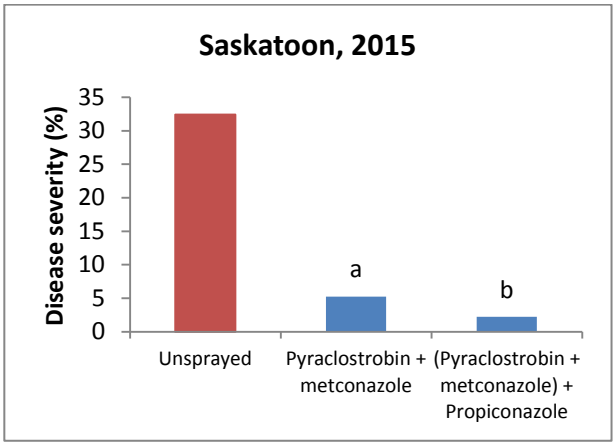

Fig. 5.25 Frequency one or two fungicide applications to control leaf mottle on canary seed at Saskatoon in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Fusarium seed infection (%)

There were no significant differences between one application of pyraclostrobin + metconazole at flag leaf or two applications of pyraclostrobin + metconazole at flag leaf stage follows by propiconazole on canary seed to control fusarium seed infection (Table 5.19).

Table 5.16 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follow by propiconazole on fusarium seed infection (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	0.1391	0.5080	0.9479
	Saskatoon	0.6924	0.8664	0.0848
2015	Indian Head	0.7140	0.9086	0.5838
	Saskatoon	0.2364	0.0729	0.3705

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

Grain yield (kg ha^{-1})

In two sites-year out of four there was an effect of fungicide treatment on canary seed yield (Table 5.20). At Indian Head in 2014, one application of pyraclostrobin + metconazole had a higher yield (1711 kg ha^{-1}) than two fungicide applications of pyraclostrobin + metconazole followed by propiconazole (1369 kg ha^{-1}) (Fig. 5.25). A contrast analysis indicated that the unsprayed check had a significantly different yield from the single fungicide application, whereas the unsprayed control was no different from treatments which had two fungicide applications. At Indian Head in 2015, Keet had higher yield, 1802 kg ha^{-1} than PI 251274-3, 1356 kg ha^{-1} (Fig. 5.26).

Table 5.17 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on yield (kg ha^{-1}) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	**0.0338**	0.1890	0.5898
	Saskatoon	0.3466	0.1591	0.4361
2015	Indian Head	0.5908	**0.0041**	0.1108
	Saskatoon	0.9433	0.8369	0.5161

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

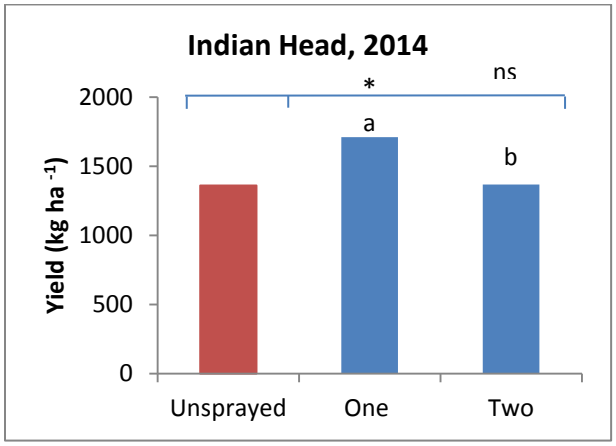

Fig. 5.26 Effect of fungicide frequency, one application: pyraclostrobin + metconazole and two applications: pyraclostrobin + metconazole follow by propiconazole on yield of canary seed at Indian Head in 2014. Contrast analysis indicates significant differences (*) between unsprayed and one, but not significant (ns) differences between unsprayed and two fungicide applications. Means with lower case letters indicate differences between one or two fungicide applications according to Tukey test ($P \leq 0.05$).

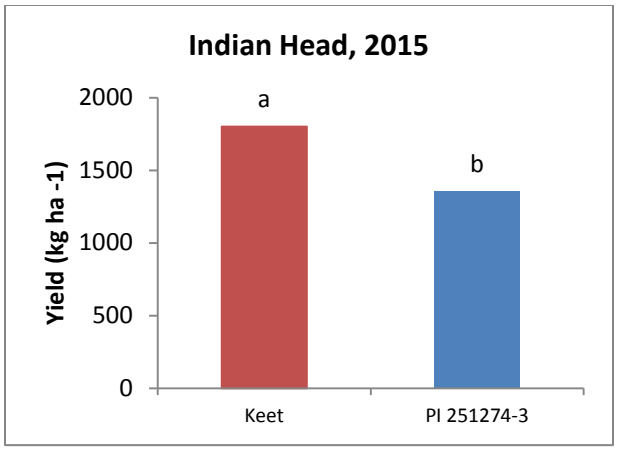

Fig. 5.27 Effect of fungicide frequency, one application: pyraclostrobin + metconazole and two applications: pyraclostrobin + metconazole follow by propiconazole on yield of two genotypes of canary seed at Indian Head in 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Thousand kernel weight (g)

In three sites-years, genotype had an effect on TKW (Table 5.21). In 2014, at Indian Head, Keet had lower a TKW (7.6 g) than PI 251274-3 (7.8 g), whereas at Saskatoon in 2014 and Indian Head in 2015, TKW was higher for Keet than for PI 251274-3 (Fig. 5.27).

Table 5.18 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on TKW (g) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	0.2495	**0.0050**	0.8643
	Saskatoon	0.2961	**0.0007**	0.8586
2015	Indian Head	0.3235	**<.0001**	1.0000
	Saskatoon	0.1920	0.8639	0.6096

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

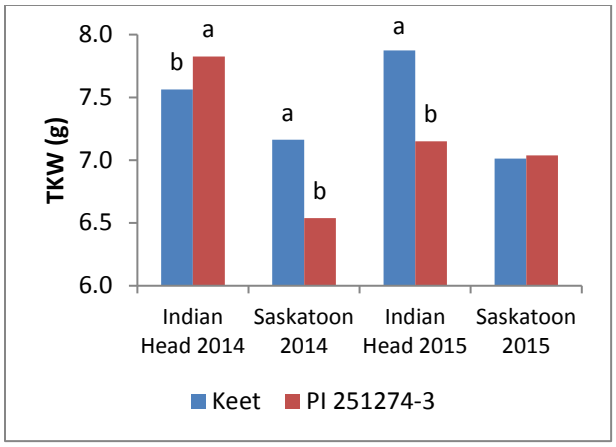

Fig. 5.28 Frequency of fungicide application and genotype on TKW of canary seed at Indian Head and Saskatoon in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

Protein content (%)

There were no significant differences between one application of pyraclostrobin + metconazole at flag leaf and two applications of pyraclostrobin + metconazole at flag leaf stage follows by propiconazole on protein content (Table 5.22).

Table 5.19 Probability of *F* values for the frequency of fungicide applications: pyraclostrobin + metconazole or pyraclostrobin + metconazole follow by propiconazole on protein content in canary seed at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	0.0553	0.1882	0.5336
	Saskatoon	0.3378	0.3039	0.8205
2015	Indian Head	0.3052	0.0995	0.4029
	Saskatoon	0.4489	0.1335	0.4134

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

Oil content (%)

In none of the four sites years was an effect of frequency of fungicide on oil content of canary seed, however in two site-years there was an effect of genotype (Table 5.23). At both locations, Keet had a lower oil content compared with PI 251274-3 (Fig. 5.28).

Table 5.20 Probability of *F* values for the analysis of variance for pyraclostrobin + metconazole or pyraclostrobin + metconazole follows by propiconazole on oil (%) at Indian Head and Saskatoon, 2014 and 2015.

Year	Location	Frequency (Fr)	Genotype (G)	FrxG
2014	Indian Head	0.9479	0.3711	0.3711
	Saskatoon	0.4311	**<.0001**	0.1158
2015	Indian Head	0.6410	**0.0002**	0.0661
	Saskatoon	0.2861	0.1901	0.7832

Degree of freedom: 1 for Fr, 1 for G and 1 for FrxG. Significant differences were indicated by ($P \leq 0.05$).

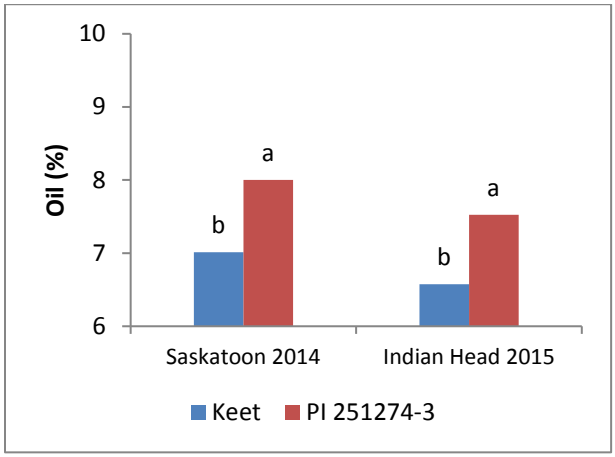

Fig. 5.29 Effect of genotype on oil content in canary seed at Indian Head and Saskatoon in 2014 and 2015. Means with lower case letters indicate differences between genotypes according to Tukey test ($P \leq 0.05$).

5.5.7 Economic analysis of fungicide application on canary seed

The economic analysis was calculated for the site years where fungicide had a significant statistically effect on yield (Table 5.24). For the flag leaf stage application, at Indian Head in 2014, the net return for pyraclostrobin + metconazole was 30% more profitable than the prothioconazole + tebuconazole. For the heading stage application at Saskatoon in 2014, the profit ha^{-1} for the propiconazole treatment was 85% more profitable than the prothioconazole + tebuconazole application. For the frequency of fungicide application, the economic analysis was calculated for Indian Head in 2014. The net return for single fungicide application was $131.41, but it was negative when two fungicides application where sprayed.

Table 5.21 Net return of fungicide application at leaf stage to control leaf mottle on canary seed at Indian Head 2014.

	Yield (kg ha^{-1})	Yield increase (kg ha^{-1})	Price (CAD $ kg^{-1})*	Gross income (CAD $ ha^{-1})	Fungicide cost (CAD $ ha^{-1}) †	Application cost (CAD $)‡	Net Return (CAD $)
Fungicide application at flag leaf stage							
Indian Head 2014							
Prothioconazole + tebuconazole	1673	311	0.51	158.61	49.54	17.30	91.77
Pyraclostrobin + metconazole	1711	349	0.51	177.99	29.28	17.30	131.41
Unsprayed	1362	-	-		-	-	-
Fungicide application at heading stage							
Saskatoon 2014							
Propiconazole	1814	480	0.51	244.80	23.48	17.30	204.02
Prothioconazole + tebuconazole	1506	172	0.51	87.72	49.54	17.30	20.88
Unsprayed	1334	-	-	-	-	-	-
Frequency of fungicide application							
Indian Head 2014							
Pyraclostrobin + metconazole	1711	349	0.51	177.99	29.28	17.30	131.41
Pyraclostrobin + metconazole follow by propiconazole	1369	7	0.51	3.57	52.76	17.30	-66.49
Unsprayed	1362	-	-	-	-	-	-

*Price of canary seed ($ kg^{-1}) Stat Publishing
†Fungicide cost (Personal communication)
‡Application cost (The Saskatchewan Ministry of Agriculture's Custom Rate Guide)

5.6 Discussion

Development of leaf mottle requires high humidity, warm temperatures, a susceptible host and a source of inoculum. In this study, variability in leaf mottle disease severity among years and sites was affected by precipitation and temperature. In 2014, average precipitation (158 mm) was 65% higher than the 30-year long-term normal at flag leaf stage (June), and temperature was similar to

the long-term normal at both locations. At the flag leaf stage, few symptoms of the disease were observed on the lower leaves of the plants. During the grain filling stage (July and August) at Indian Head, precipitation was higher than the long-term normal. At Saskatoon precipitation was similar to the long-term normal and symptoms were observed on the upper leaves of the plants. As a result of the weather conditions in 2014, plants were tall with some lodging at the end of the growing season. In contrast, in 2015, precipitation (33 mm) was 35% lower than the long-term normal at the flag leaf stage (June) and 77% lower than in 2014. Temperature was higher at Indian Head (24.5^{o}C) than at Saskatoon (18.5^{o}C) in June, 2015. Due to the dry conditions in 2015, plants started flowering earlier at Saskatoon than at Indian Head. At the flag leaf stage, plants were very short and there were no symptoms of leaf mottle, whereas at Indian Head flowering was not affect by the dry conditions. During the heading stage (July and August), precipitation was 60% higher than in June and 37% lower than the long-term normal, which was conducive to the development of leaf mottle later in the season. In general, dry conditions in 2015 limited both diseases, leaf mottle and fusarium seed infection, compared with 2014. Although symptoms of fusarium seed infection in the panicle of canary seed was not easy to identify in the field, most of seed was found to be infected when the seed was plated on agar media in the laboratory.

Each of the treatments: two fungicides products, two fungicide timing applications and two canary seed genotypes, had an effect on leaf mottle, even when disease severity was moderate. For example, in 2014 leaf mottle severity of the unsprayed plots (average of both canary seed genotypes) was 33%, but there was no interaction among treatments. In contrast, in 2015, when disease severity was very low (<7% on the unsprayed check and <1.5% in sprayed treatments), the interaction between application timing and genotype was significant, although this result was not biologically relevant because the low disease severity had little impact on yield or quality.

Prothioconazole + tebuconazole (Prosaro ®) often had an effect on leaf mottle of canary seed when applied at either flag leaf or heading stages, compared with propiconazole. The Fungicide Resistance Action Committee (FRAC) (2015) classified the active ingredients of these two fungicides into the same group: demethylation inhibitors (DMI) fungicides, with a common mechanism of action, the inhibition of sterol biosynthesis. Within this group there are two sub groups, triazoles and triazolinthiones. Propiconazole is a triazole and prothioconazole is a triazolinthione, the combination of the two actives improved effectiveness of leaf mottle control compared with a single active ingredient. The effect of timing was a challenge to measure due to the short period between flag leaf and heading stage and also the variability between the two canary seed genotypes, because PI 251274-3 flowered earlier than Keet. However, the mixture of prothioconazole + tebuconazole provided better control due to the combination of the two active ingredients in one product and the greater systemic effects of tebuconazole than propiconazole.

Integration of two fungicide products, two fungicide timing application and two canary seed genotypes resulted in differences in leaf mottle disease severity between genotypes; Keet had lower disease severity than PI 251274-3. PI 251274-3 was expected to be moderately resistant to leaf mottle based on testing under controlled conditions using a single isolate of *S. triseti* (Hucl et al., 2014). However, this was not observed in this study; PI 251274-3 suffered higher disease severity than Keet, which was expected to be susceptible to leaf mottle. One explanation for this observation was that isolate used for the indoor study was not representative of the population present at the two locations of this study. Another reason could be that canary seed may have two types of leaf mottle resistance, seedling or race-specific resistance and adult stage or race-nonspecific resistance. In a study of 48 accessions, 47 were susceptible at the seedling stage; however, when nine of these accessions were re-tested at the adult plant stage, seven were

moderately resistant, indicating that canary seed may carry adult plant resistance (Hucl et al., 1997). The same types of resistance occur in wheat when challenged by *Mycosphaerella graminicola*; some varieties of wheat are susceptible to this pathogen at the seedling stage, but not at the adult plant stage, and vice versa (Kema and Van Silfhout, 1997; Cowger et. al., 2002). Canary seed genotype PI 251274-3 was susceptible at both stages. Also, studies in wheat suggested that genotypes with longer maturity are resistant, whereas genotypes with shorter maturity are more susceptible (Rodrigo et al., 2014). In this study, PI 251274-3 always headed earlier than Keet. This may also have influenced disease development on PI 251274-3, compared with Keet.

From the two fungicide products, two fungicide timing application and two canary seed genotypes, fungicide was the most important factor affecting fusarium seed infection in 2014 when infection was 12% (average over both canary seed genotypes). Similar to leaf mottle, fusarium seed infection was best controlled by prothioconazole + tebuconazole at heading stage, compared with propiconazole. Differences among DMI fungicides were reported on germination of ascospores and radial mycelial growth of *F. graminearum* (Wallhead et al, 2007 and Klix, 2007). In canary seed, differences in yield were related to genotype, rather than to fungicide product or fungicide application timing. However, differences in yield of the genotypes were not consistent among sites-years. In 2014, PI 251274-3 out yielded Keet at one site, but the opposite occurred in 2015 at one site. High variability of yield has been reported for canary seed due to soil characteristics (Hucl et al., 1997), drought conditions, seeding date (IHARF, 2013; May et al., 2012), and day length sensitivity (Xyntaris, 2015). This indicates that some genotypes may perform differently due to agronomic and environmental conditions.

Fungicide products and canary seed genotypes had various effects on seed quality. Often TKW was higher for Keet than PI 251274-3, which may have been due to early flowering of PI 251274-3, compared with late flowering of Keet. A genotype with early flowering can be infected over a longer period than a late flowering genotype, which was the case for Keet in this study, but also the difference in TKW could be an inherent characteristic of each genotype.

The protein content of PI 251274-3 was usually, but not always higher than that of Keet. Cultivar differences were observed for wheat protein content, which was affected more by cultivar than by fungicide application (Monaghan, et al., 2001). For oil content, the interaction of fungicide products, fungicide timing and canary seed genotype indicated that applications of prothioconazole + tebuconazole at heading stage may result in reduced oil content of canary seed. Fungicide application at the heading stage may result in lower oil content than at the flag leaf stage, and oil content in Keet was lower than in PI 251274-3. The effect of prothioconazole + tebuconazole on oil content of rapeseed indicated that oil content was enhanced when prothioconazole + tebuconazole or azoxystrobin was applied during pod filling stage as a result of reduced lodging, which was related to canopy density (Ijaz and Honermeier; 2011).

Application of three fungicides at flag leaf stage indicated that prothioconazole + tebuconazole or pyraclostrobin + metconazole controlled both leaf mottle and fusarium seed infection at one site-year, whereas propiconazole did not. Differences in the control of leaf mottle could be due to differences in environmental conditions. Similar results were observed in canary seed at Stewart Valley in 1999, 2000 and 2001, and Indian Head in 2001, when leaf mottle disease severity was light, there was no response to the application of propiconazole (May, 2001). At least one active ingredient of each fungicide belongs to the azole group: tebuconazole, propiconazole and prothioconazole and among them, prothioconazole has strong translaminar movement (Klittich

and Ray, 2013), which results in reduced fungal infection of the leaves and may indicate why prothioconazole + tebuconazole was more effective to control both diseases. Pyraclostrobin + metconazole increased yield in dry conditions in the absence of disease or under low disease severity (<7% on the unsprayed check averaged over the two genotypes). This indicated that the increased yield of canary seed may be related to the physiological effect of strobilurins on the plant. When plants are stressed by dry conditions, loss water in the plant is regulated via stomata closing and decreased water loss, thus a delay in crop maturity occurs, which allows for more physiological activity during the grain filling stage (Wu and Tiedemann, 2001). However, fungicide treatments applied to canary seed at the flag leaf stage did not affect TKW, protein or oil content. A numbers of studies on a number of crops have reported that fungicide application has no effect on yield, kernel weight, test weight or protein content under dry conditions (Wang et al., 2002; Blandino and Reyneri, 20009).

Fungicides sprayed at the heading stage had little to no effect on leaf mottle or fusarium seed infection. Prothioconazole + metconazole controlled fusarium seed infection (P<0.05) in 2014, compared with propiconazole, but only in one site-year. This indicated that applications of prothioconazole + metconazole at the heading stage did reduce fusarium seed infection to 2.4%, compared with propiconazole at 9.6%. Similarly, on spring wheat when disease appears late in the season, fungicide application is recommended at the beginning of anthesis rather than at the flag leaf stage (Wiersma and Motteberg, 2005), which seems to be similar in the control of leaf mottle of canary seed. PI 251274-3 had lower leaf mottle than Keet in one site-year, but it had higher fusarium seed infection at both locations. This suggested that PI 251274-3 was more susceptible than Keet to fusarium seed infection. Genotype had a greater effect on yield at two site-years. This suggested that yield was more affected by genotype than by fungicides. Keet

yielded higher than PI 251274-3 in 2015, but there was no difference in 2014. This suggested that yield of Keet may have been related to the dry conditions in 2015, rather than high disease severity in 2014.

Fungicide application at heading stage did not have any effect on TKW of canary seed, whereas genotype did affect TKW. Thousand kernel weight of Keet was higher than that of PI 251274-3 in three site-years. This may have occurred because PI 251274-3 flowered earlier than Keet, and when leaf mottle severity and fusarium seed infection were high, it affected filling, thus TKW. However, in 2014, the occurrence of lodging of Keet may have been responsible for reduced TKW of Keet, compared with PI 251274-3.

Protein content was slight lower when prothioconazole + tebuconazole was applied at heading stage, compared with propiconazole, but it was not different from the unsprayed treatment. This indicated that fungicide products did not have a strong effect on protein in canary seed, but differences in protein content were related to genotype; PI 251274-3 had higher protein content when fungicide was applied at heading stage than the unsprayed treatment.

Oil content was lower in unsprayed treatments compared with the sprayed treatment. This indicated that fungicide can increase oil content in canary seed. Similar effects have occurred in some oil crops, such as canola, where application of azoxystrobin (Ortiva ®) and boscalid (Cantus ®) in combination with triazole fungicides enhanced oil content by extending the seed formation phase, which led to increased oil accumulation in the seeds (Ijaz and Honermeier, 2011). However, oil content was reduced by fungicides at one site-year when fungicides were applied at heading stage; prothioconazole + tebuconazole reduced the oil content. PI 251274-3 had higher oil content

than Keet in 2015. In general, genotype had the greatest effect on oil content of canary seed. PI 251274-3 had higher protein and oil contents, but lower TKW and yield.

It was expected that pyraclostrobin + metconazole at the flag stage followed by propiconazole application at the heading stage would prolong disease control compared with one application of pyraclostrobin + metconazole at the flag stage. However, two applications of fungicide reduced leaf mottle disease severity only in 2015. An interaction between frequency and genotype suggested that a single application of fungicide at flag leaf stage controlled leaf mottle on Keet, whereas, two fungicide applications were more effective on PI 251274-3. Frequency of fungicide application did not have any effect on fusarium seed infection. It has been report that strobilurins and propiconazole has little or no effect on FHB in spring wheat and barley (Simpson et al., 2001; Hollingsworth et al., 2006). This suggests that one application of pyraclostrobin + metconazole at flag leaf stage could control leaf mottle but not fusarium seed infection and two applications of fungicides did not reduce fusarium seed infection of canary seed. However, a single application of pyraclostrobin + metconazole increased yield by 20% compared with both the unsprayed and the dual fungicide application treatments.

Genotype was the only treatment that affected TKW, PI 251274-3 tended to have a higher TKW than Keet. Frequency of fungicide products did not affect oil content, but PI 251274-3 had a higher oil content than Keet, which may have been due to an inherently higher genetic potential for oil content.

In general, profit should increase when fungicides control high levels of disease severity and this effect should translate into a yield response. In this study, all fungicide treatments had a higher yield than the unsprayed treatments, although the results were not positive enough to justify

fungicide as a means to increase yield. Prothioconazole + tebuconazole provided the best control of leaf mottle and fusarium seed infection; however it was more profitable to apply this fungicide at the flag leaf stage ($91.77) than at the heading stage ($20.88). The profitability of this fungicide was higher due to the greater yield response at flag leaf. However, yield response in canary can be highly variable due to environmental conditions, inherent genetic yield potential, fertility, soil characteristics or other physiological processes. Among the three fungicides evaluated in this study, propiconazole ($204.02) resulted in a higher economic return, although it was not the most effective against leaf mottle or fusarium seed infection. The advantage of propiconazole was related to its low cost, rather than a consistent increase in yield, control of fusarium seed infection or leaf mottle. Two fungicide applications were less profitable compared with single application, due to the price of the fungicides. However, the yield increase of this treatment did not offset the additional cost of the two fungicide applications. It would require an additional yield of 342 kg ha^{-1} to cover the cost of the products at current canary seed prices. It was observed that the dual applications had a similar net return to a single fungicide application.

5.7 Conclusion

In this study, leaf mottle of canary seed was affect by fungicide product, fungicide timing, genotype and frequency of fungicide application, whereas fusarium seed infection was affected by fungicide product only. Application of prothioconazole + tebuconazole when disease severity was >30% in unsprayed treatments reduced leaf mottle and fusarium seed infection. Unsprayed treatments had higher levels of leaf mottle and fusarium seed infection than sprayed treatments most of the time, but there was little effect on yield, TKW or protein or oil content. Considering the presence of fusarium seed infection on canary seed and late leaf mottle disease development, one application of prothioconazole + tebuconazole at heading stage may control both diseases. If

conditions are suitable for severe disease development, fungicides should be applied at flag leaf stage, but are not needed in dry years. The increased net return (after cost of fungicide and application) of prothioconazole + tebuconazole ranged between $20.88 and $91.77 ha^{-1}. However, the final decision by growers should be based on the susceptibility of the genotype, and the potential for severe disease development, which includes an awareness of the weather conditions prior to flag leaf or heading stages.

CHAPTER 6:

General discussion and future research

6.1 Discussion and conclusion

Leaf mottle is reported to be the most important disease of canary seed (Berkenkamp et. al., 1989). Successful management of this disease includes development of resistant varieties and fungicide application. Canary seed was approved as food for human consumption and therefore identification of pathogenic fungal species on canary seed panicles is necessary to monitor seed quality. This thesis examined interactions between *Septoria triseti* isolates and canary seed genotypes and provided evidence that this pathosystem follows the gene-for-gene paradigm (Chapter 3). Identification of *Fusarium graminearum* on canary seed in Saskatchewan during 2014 and 2015 (Chapter 4) was useful to understand this pathogen in the province and to implement appropriate integrated pest management (IPM) strategies. Fungicide application reduced leaf mottle severity and fusarium seed infection of canary seed (Chapter 5). A decision to apply fungicide at flag leaf should consider the local environmental conditions and the crop growth stage when disease is detected; however, in this study it was difficult to measure the effect of fusarium infection on the canary seed head under field conditions. Canary seed genotypes varied in terms of yield, TKW, and protein and oil content. The cultivar Keet tended to have higher yield and TKW, however, the breeding line PI 25127-3 higher oil and protein content. Application of pyraclostrobin + metconazole at the flag leaf stage follow by application of propiconazole at heading stage resulted in a negative net economic return. Prothioconazole + tebuconazole was the

most effective fungicide combination to control both diseases; fungicide increased profit as much as \$91.77 ha^{-1}, whereas, two separate applications of two fungicides, required an additional yield increase of 342 kg ha^{-1} to cover the cost of the products and two applications to result in a similar net return as one fungicide application. However, the variability in yield and late onset of disease may be two factors that influence fungicide profitability.

6.2 Future studies

Detection of gene-for-gene interactions of *S. triseti* and *Phalaris* spp. suggests the presence of race-specific resistance and therefore one or more resistance genes with major effect. Increasing the number of isolates can give a better understanding of the pathotypes of *S. triseti*. Phenotyping of adult plants is important to understand adult plant resistance in canary seed. Epidemiological studies of *S. triseti* are necessary to understand the behavior of leaf mottle disease in the field. For the development of leaf diseases such as those caused by *S. tritici*, *S. nodorum* and other *Septoria* spp. in wheat, an understanding of the interactions of plant growth, rain splash and the availability of inoculum is important. Studies of canary seed have been conducted under the assumption that the highest risk to the plant is at flag leaf stage. However, the size of the flag leaf is small in canary seed, which makes it unsuitable for evaluation of leaf mottle severity, and the role of the flag leaf on yield of canary seed is likely limited. Therefore, the benefit of late fungicide application without consideration of the flag leaf should be tested. This characteristic could be similar to barley in which the flag leaf is less important since it is small and lower leaves have greater photosynthetic efficiency during grain filling.

Late appearance of leaf mottle disease severity was observed in wet and dry years. Less yield loss was reported when the disease appeared late and greater loss when the disease appeared early. This study, in which one year was considered wet and the other dry, was not sufficient to determine

the effect of the disease on yield and quality of canary seed. Therefore, additional years and sites of experimentation should be considered.

Since canary seed has been accepted as safe for human consumption, the presence of *Fusarium graminearum* on canary seed is important. Studies of mycotoxin content and examination of the relationship between incidence and severity are suggested to ensure product safety and quality. Also, studies of the infection process of this pathogen are necessary to better understand the development of fusarium seed infection to integrate fungicide control in leaf mottle and fusarium seed infection management of canary seed. It would be prudent to determine the impact of fungicide application on DON levels and the mycotoxins produced by *F. graminearum* on canary seed. There are challenges in the identification of symptoms of fusarium seed infection of canary seed in the field. Most of the seed infected was not observed by visual observation, however, as a result of test plating of seed high levels of seed infection were detected. Studies of the infection process using microscopy techniques would clarify the biological interaction of *F. graminearum* on canary seed to be able to integrate alternative methods for applying fungicides in the field. Further research is necessary to better understand the effects of fusarium seed infection on yield and seed quality of canary seed. In wheat, FHB is caused by a complex of *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides* (Parry et. al, 1995). For this reason it is important to take into account the distribution and prevalence of *Fusarium* spp. and the mycotoxins produced. Strobilurin fungicides have poor efficacy against FHB although these fungicides have been reported to increase the DON mycotoxins (Simpson et. al., 2001).

In conclusion, this study provided important information that could be used to improve the management of leaf mottle and fusarium seed infection of canary seed. First, the characterization

of the *Septoria triseti - Phalaris canariensis* pathosystem identified one genotype of *P. canariensis* that that was highly resistant to leaf mottle and might be included in the breeding program. Second, the identification of pathogenic fungal species in canary seed and the first report of *F. graminearum* on canary seed suggested that it is necessary to include fusarium control strategies in canary seed. This would be a starting point for further epidemiological, breeding and agronomic studies. Finally, the fungicide study suggested that leaf mottle disease is related to yield losses in canary seed and fungicides need to be applied with consideration of environmental conditions and canary seed genotype.

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APPENDICES

Appendix 1. Survey report of disease of canary seed in Saskatchewan 2014.

CROP / CULTURE: Canary seed (Phalaris canariensis)
LOCATION / RÉGION: Saskatchewan
NAMES AND AGENCIES / NOMS ET ÉTABLISSEMENT:
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TITLE / TITRE: DISEASE OF CANARY SEED IN SASKATCHEWAN

ABSTRACT: *Septoria triseti* Speg., cause of leaf mottle, and three *Fusarium* spp. on seed were the most frequently isolated pathogens from canary seed (*Phalaris canariensis*) in Saskatchewan in 2014. Most of the 21 crops sampled from the southeeast and southwest regions of the province were affected by both leaf mottle and *Fusarium* at varying levels depending on location.

INTRODUCTION AND METHODS: A survey to document the diseases affecting canary seed crops in Saskatchewan was conducted from August 12 – 24, 2014. The 21 randomly-selected crops varied in maturity between BBCH growth stages 65 and 89 (full flower to maturity; Lancashire *et al*. 1991). Leaf mottle severity was assessed on the flag-1 and flag-2 leaves as the percentage of the leaf area affected (Horsfall, *et al*. 1945). The average severity on the two leaves was categorized as: trace (0-10%), light (6-10%), moderate (11-40%) and severe (41-100%). Leaves with leaf mottle symptoms (necrotic tissue with black pycnidia) were collected from each crop and dried in paper envelops. Subsequently, affected tissue pieces from 10 leaves per crop were surface-sterilized in 70% ethanol for 1 min and then rinsed 3 times in sterile water. The leaf tissue pieces were plated on water agar containing streptomycin for 3 days after which the proportion of these harboring the leaf mottle pathogen, *Septoria triseti*, was determined by visual observation. To determine the occurrence and level of seed infection, 100 seeds from each crop were surface-sterilized in 70% ethanol for 1 min, rinsed 3 times in sterile water, and then vacuum dried. Seeds were plated on PDA (potato dextrose agar) and incubated under 12 h light/dark at room temperature for 6 days (Warham, *et al*. 1995). Morphological keys were used to identify the species of *Fusarium* present (Gerlach and Nirenberg 1982). Prevalence of *Fusarium* was determined by counting the numbers of crops affected by *Fusarium* spp., and incidence was calculated from the proportion of seeds infected with *Fusarium* spp.

RESULT AND CONCLUSIONS: Among the 21 crops surveyed, *S. triseti* was observed in 15 crops for a prevalence of 71.4%. Six crops were free of leaf mottle, and may have been sprayed with fungicides; severity levels in the others were trace – 4 crops, light – 1, moderate – 10 (Table 1). The incidence of *S. triseti* from the 210 leaf tissue pieces tested in the laboratory was 49%. In addition to leaf mottle, aphids were observed in many canaryseed crops and some lodging was noted. Lodging was more prevalent in the southeast of the province compared to the southwest, possibly due to greater precipitation in the former region.

Prevalence of *Fusarium* in the 21 canaryseed crops was 95%; only one crop was *Fusarium*-free. The three species identified were *F. graminearum, F. avenaceum* and *F. equiseti*, at a prevalence among crops of 90%, 48% and 14%, respectively (Table 2). The incidence *F. graminearum*- infected seed among the crops was as high as 73% (Table 3). The highest incidences of *F. avenaceum* and F. equiseti on seed ware 8% and 7%, respectively. Other fungi observed occasionally included *Alternaria* spp. and *Bipolaris* spp.

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Table 1. Severity of leaf mottle in canary seed crops in Saskatchewan, 2014.

6.3 Severity level	6.4 % leaf area affected	# Crops	Severity (%)
None	0	6	29
Trace	1 – 5	4	19
Light	6 – 10	1	5
Moderate	11 – 40	10	48
Severe	41 – 100	0	0

Table 2. *Fusarium* spp. isolated from seed of canary seed in Saskatchewan in 2014.

	% Affected Crops	% of Kernels*
Total *Fusarium* spp.	95	14
Fusarium graminearum	90	12
Fusarium avenaceum	48	2
Fusarium equiseti	14	0.4

***** Based on a total of 2,100 seeds.

Table 3. Incidence of *Fusarium* spp. in 21 crops of canary seed in Saskatchewan, 2014.

7 Crop #	SK Crop District #	*Fusarium graminearum* (%)	*Fusarium avenaceum* (%)	*Fusarium equiseti* (%)
1	2B	2	0	0
2	2B	3	2	1
3	2B	3	0	0
4	2B	17	3	0
5	2B	5	1	0
6	2B	8	0	0
7	2B	5	1	0
8	8B	73	0	0
9	8B	2	0	0
10	4B	4	2	0
11	4B	1	0	0
12	7A	0	0	1
13	7A	5	5	0
14	7A	24	0	0
15	7A	9	6	0
16	7A	0	0	0
17	7A	34	8	0
18	7A	20	2	0
19	7A	27	5	7
20	5B	1	0	0
21	5B	1	1	0

Appendix 2. Survey report of disease of canary seed in Saskatchewan 2015.

CROP / CULTURE: Canaryseed
LOCATION / RÉGION: Saskatchewan

NAMES AND AGENCY / NOMS ET ÉTABLISSEMENT:
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TITLE / TITRE: DISEASES OF CANARYSEED IN SASKATCHEWAN IN 2015

ABSTRACT: Leaf mottle caused by *Septoria triseti* was observed in canaryseed (*Phalaris canariensis*) crops and *Fusarium* spp. detected in seed of these crops in Saskatchewan in 2015. Leaf mottle was observed in 78% of crops, and severity was at a trace level in most of these. Prevalence of *Fusarium* spp. was 88% with three species identified: *Fusarium graminearum*, *F. avenaceum* and *F. poae*. Incidence of *F. graminearum* on seed averaged 3% over the 26 crops, and was lower for *F. avenaceum* and *F. poae*.

INTRODUCTION AND METHODS: Twenty-three canaryseed crops were sampled randomly for leaf mottle in early August and 26 crops for *Fusarium* spp. during growth stages BBCH 65 - 89 (full flower - maturity) (Lancashire et al. 1991). Ten leaves taken from the upper canopy were assessed for leaf mottle on a 0 – 5 severity scale: trace (<1% (of leaf tissue affected), very slight (1-5%), slight (6-15%), moderate (16-40%) and severe (41-100%) (Horsfall and Barratt 1945). Leaves with (or without) leaf mottle symptoms (necrotic tissue with black pycnidia) were collected from each crop and dried in paper envelopes. Subsequently, a piece from each of the 10 leaves was surface-sterilized in a solution of 5% NaOCl for 1 min and then rinsed three times in sterile water. The leaf pieces were plated on sterile filter paper, and after 24 hours the percentage of the leaf pieces that harbored the leaf mottle pathogen was confirmed by visual observation. To test for the presence of *Fusarium* spp., 100 seeds per field (2,600 total) were surface sterilized in 5% NaOCl for 1 min, rinsed three times in sterile water and then dried. Seeds were plated on PDA (potato dextrose agar) and placed under a 12 hour light/dark regime at room temperature for 5 days (Warham at al. 1995). *Fusarium* species present were determined by the shape and size of their macrospores (Gerlach and Nirenberg 1982). Prevalence of *Fusarium* spp. was determined by counting the proportion of crops affected, and incidence by counting the number of seeds affected by each *Fusarium* sp. from the 100 plated for each canaryseed crop.

RESULTS AND CONCLUSIONS: Among the 23 crops surveyed, *Septoria triseti* Speg.was observed in 18, giving a prevalence of 78%. Fifteen of the 18 crops were determined to have a trace of leaf mottle, two had very slight, and one had slight severity (Table 1). The incidence of *S. triseti* on the 10 leaves collected from each crop (230 leaves total) was 16%.

The prevalence of all *Fusarium* spp. on the 2600 canaryseed seeds examined was 88% (Table 2). Only three fields were *Fusarium*-free. Three species were identified: *F. graminearum*, prevalent in 58% of the 26 crops, *F. avenaceum* in 50% and *F. poae* in 35%. Averaged over all 26 crops, the incidence of *F. graminearum* on seed was 3%, *F. avenaceum* 1% and *F. poae* 1%. The incidence of *F. graminearum* on seed varied among crops from 29% in one crop to zero in 11 crops (Table 3). Other fungi were detected on leaf pieces and seed, such as *Alternaria* spp., but were considered to be saprophytes.

In addition, aphids were observed in many canaryseed crops, while lodging was minimal.

ACKNOWLEDGEMENTS:

We thank X.M. Zhang for sample collection and help with identification of *Fusarium* species, and to the CFPATH group for the survey coordination.

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Table 1. Severity of leaf mottle in 23 Saskatchewan canaryseed crops in 2015.

Disease severity	Number of crops	Proportion of crops in each category (%)
None	5	22
Trace	15	65
Very slight	2	9
Slight	1	4
Moderate	0	0
Severe	0	0

Table 2. Prevalence and incidence of *Fusarium* spp. in 26 Saskatchewan canaryseed crops, in 2015.

	Prevalence[1] (%)	**Incidence[2] (%)**
Total *Fusarium* spp.	88	6
Fusarium graminearum	58	3
Fusarium avenaceum	50	1
Fusarium poae	35	1

[1]Proportion of crops with *Fusarium* spp.

[2]Based on a 100 seed sample per crop

Table 3. Incidence (%) of *Fusarium* spp. on 100-seed samples of canaryseed from 26 Saskatchewan crops in 2015.

Field #	Crop District	*F. graminearum* (%)	*F. avenaceum* (%)	*F. poae (%)*
1	2B	2	0	2
2	2B	29	2	0
3	2B	1	0	0
4	2B	0	2	3
5	2B	0	2	1
6	2B	0	0	0
7	4B	0	1	0
8	4B	2	0	0
9	4B	0	0	0
10	4B	1	0	0
11	4B	0	2	3
12	7A	0	0	1
13	7A	0	0	0
14	7A	0	0	0
15	7A	0	0	0
16	7A	18	1	0
17	8B	4	0	2
18	5B	1	2	3
19	5B	3	2	0
20	5B	2	1	0
21	5A	1	3	2
22	2B	2	0	0
23	2B	2	1	0
24	2B	0	0	1
25	2B	1	1	0
26	2B	3	2	0

Appendix 3. Note disease publish in the journal Plant Disease

First report of Fusarium head blight, caused by *Fusarium graminearum*, on Annual Canarygrass (*Phalaris canariensis*) in Saskatchewan, Canada.

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Annual canarygrass or canary seed (*Phalaris canariensis* L.) is currently used for feeding caged birds, but it recently achieved generally regarded as safe (GRAS) status for human consumption. Fusarium head blight (FHB) caused mainly by members of the *Fusarium graminearum* species complex causes considerable losses in grain quality and yield loss of wheat, oat and barley. In August, 2014 in Saskatchewan, Canada, symptoms of FHB were observed in commercial annual canarygrass fields. The panicles appeared bleached and prematurely ripened, with orange sporodochia and mycelium on the glumes. Twenty-one canarygrass fields were surveyed from 5 crop districts across the province of Saskatchewan. Twenty heads were collected from each field during growth stages BBCH 65 - 89 (full flower - maturity) (Lancashire et al. 1991) and threshed. One hundred seeds from each sample were randomly selected, surface sterilized in 70% of ethanol for 1 min, rinsed 3 times with sterilize water, and vacuum dried. Seeds were plated on PDA and incubated under 12 h light/dark at room temperature for five days. From the 21 fields, *F. graminearum* was identified in 19, prevalence of 90% and from the 2100 seeds plated, the pathogen was isolated from 252 seeds, incidence of 12%. Colonies of *F. graminearum sensu lato* were identified based on morphological characteristics, including color, absence of microconidia and size of spores (Gerlach and Nirenberg, 1982). Eight isolates were selected for molecular identification. *Fusarium graminearum* single-spore cultures were prepared and mycelia were cultured in liquid medium for five days, harvested, vacuum dried and ground in liquid nitrogen. The DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN®, Germany). Primers and a TaqMan probe (6-FAM/TAMPRA) specific to *F. graminearum* used were designed by Yli-Mattila et al., (2008). Real-time PCR was performed to confirm the identity of the isolate. Real-time PCR reactions were carried out in 10 µL reaction volumes, containing 1 µL DNA template, 100 nM of each primer and probe and 5 µL of Master Mix. All the results from all three replications were positive for *F. graminearum sensu lato*.

One isolate (14FG01) from one field located at Kindersley (Saskatchewan 51°14'17.9''N/108°49'08.2''W) was used to prove Kochs' postulates. A randomized complete block design experiment of four replications was conducted using cultivar Keet, which was seeded in pots with three seeds per pot (one replication), and placed in a growth chamber at 22°C day / 18°C night and a 16 h photoperiod. Canarygrass panicles at 50% anthesis were spray inoculated with either a spore suspension (5×10^4 ml^{-1}) of isolate 14FG01 or sterilized water (controls). The first visible symptoms, lesions and mycelium, on the panicles appeared four days after inoculation (dai); at seven dai some panicles appeared bleached and the peduncle tissues were brown. No symptoms appeared on the panicles of the controls. Plants were harvested 42 dai and six panicles per replication were threshed individually. Prematurely ripened seeds were very common on inoculated panicles, but not on the panicles of control plants. Prematurely ripened seeds were separated from healthy seeds. Healthy seeds were hulled, seed from treated plants were discolored and some were highly shrivelled, whereas seeds from the control were plump, of normal color (dark brown) with no visual infection symptoms. The hulled seeds were weighed, and 400 seeds were randomly chosen for re-isolation and to test for incidence of *F. graminearum*. The average incidence of seed infected by *F. graminearum* was 28%. This is the first report of *F. graminearum sensu lato* in canarygrass in

Saskatchewan. Identification the species associated with *F. graminearum* is necessary as the first step to develop strategies for management of this fungus on canarygrass.

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Appendix 4. Means and SEM of 27 isolates (Septoria triseti) x 23 genotype (*Phalaris canariensis*) and 1 genotype of (*Phalaris brachystachys*).Yellow color indicates resistance response (≤2) and white susceptible response (>2).

	PI380967		PI189547		PI203913		PI250741		Calvi		Bastia		PI163357	
Isolates	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
13_LM09	1.00	0.58	3.56	0.63	3.63	0.58	4.06	0.56	4.13	0.41	4.25	0.40	4.13	0.59
14_LM04	0.25	0.25	2.38	0.68	2.00	0.54	4.06	0.43	3.79	0.31	3.75	0.48	4.13	0.43
07_LM02	0.63	0.24	1.50	0.23	2.31	0.24	2.88	0.48	2.75	0.25	3.06	0.06	3.06	0.37
07_LM03	0.38	0.24	1.06	0.06	2.81	0.45	3.75	0.27	3.31	0.28	2.75	0.25	3.38	0.55
07_LM04	0.50	0.29	1.63	0.48	3.31	0.21	3.75	0.63	2.88	0.31	3.00	0.42	3.38	0.24
07_LM05	0.42	0.22	1.63	0.22	2.81	0.33	3.75	0.47	3.88	0.26	3.44	0.19	3.81	0.37
13_LM06	1.56	0.70	1.81	0.49	3.31	0.87	4.00	0.47	4.13	0.52	3.75	0.75	4.31	0.40
13_LM07	0.50	0.50	2.00	0.84	3.00	0.71	4.13	0.13	4.29	0.17	3.88	0.31	4.50	0.29
13_LM08	0.75	0.14	1.25	0.66	3.00	0.41	3.19	0.28	3.00	0.41	3.44	0.26	3.50	0.29
13_LM10	0.38	0.24	1.81	0.56	3.31	0.43	4.31	0.43	3.77	0.35	3.81	0.41	4.44	0.48
14_LM01	0.50	0.10	1.25	0.58	2.56	0.26	3.88	0.22	3.13	0.13	3.19	0.19	3.44	0.26
14_LM02	0.63	0.22	1.13	0.43	2.88	0.68	3.69	0.19	3.50	0.29	3.75	0.28	3.50	0.20
14_LM03	0.56	0.24	1.75	0.25	3.75	0.48	4.25	0.40	3.88	0.58	4.00	0.31	4.00	0.41
14_LM05	0.38	0.24	1.94	0.31	2.75	0.48	3.56	0.45	3.50	0.35	4.71	0.17	4.25	0.32
14_LM06	0.13	0.07	1.50	1.17	4.13	0.48	3.88	0.97	3.88	0.72	4.46	0.21	4.56	0.19
14_LM07	0.94	0.31	1.50	0.20	3.19	0.55	4.50	0.35	4.75	0.25	4.63	0.22	4.31	0.43
14_LM08	0.25	0.18	0.81	0.12	2.81	0.74	3.94	0.54	3.31	0.55	4.00	0.00	3.56	0.33
14_LM10	0.56	0.26	1.25	0.42	3.44	0.28	4.44	0.19	4.13	0.33	4.25	0.25	3.94	0.66
07_LM01	0.38	0.24	0.75	0.31	1.44	0.28	3.19	0.19	2.56	0.21	2.38	0.24	3.06	0.46
13_LM02	1.13	0.31	0.81	0.28	1.44	0.30	3.31	0.44	2.25	0.10	2.90	0.34	3.19	0.28
13_LM03	0.75	0.25	1.75	0.48	2.00	0.10	2.81	0.30	2.98	0.28	3.31	0.24	3.31	0.24
14_LM09	0.25	0.10	0.69	0.28	2.00	0.44	3.19	0.91	2.88	0.44	2.85	0.33	2.56	0.79
14_LM13	0.38	0.24	0.69	0.30	1.75	0.18	2.88	0.24	2.69	0.43	2.94	0.33	3.50	0.27
13_LM05	1.44	0.50	0.88	0.43	1.94	0.06	2.38	0.26	2.31	0.28	2.19	0.28	2.13	0.07
14_LM12	0.13	0.13	0.31	0.24	1.19	0.28	2.88	0.43	2.56	0.33	2.44	0.50	2.44	0.58
14_LM11	0.19	0.12	0.38	0.07	1.88	0.13	3.19	0.49	2.65	0.65	1.88	0.22	1.69	0.43
13_LM04	0.13	0.13	0.69	0.43	1.13	0.41	1.69	0.28	1.94	0.06	2.25	0.25	2.06	0.16

	PI284180		Cantate		PI251274		C05041		Elias		Keet		Maria	
Isolates	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
13_LM09	4.25	0.48	4.00	0.35	4.25	0.48	4.13	0.52	4.04	0.50	4.50	0.29	3.94	0.66
14_LM04	3.94	0.36	3.50	0.55	3.75	0.40	3.67	0.41	2.75	0.63	4.25	0.48	3.81	0.37
07_LM02	3.06	0.06	2.15	0.26	3.50	0.29	2.56	0.26	2.75	0.10	3.06	0.06	2.56	0.26
07_LM03	3.63	0.53	3.17	0.29	3.06	0.54	4.00	0.41	3.46	0.38	3.38	0.16	3.42	0.58
07_LM04	3.38	0.24	3.25	0.44	3.27	0.38	2.31	0.34	2.81	0.45	2.94	0.41	3.25	0.25
07_LM05	3.13	0.13	3.50	0.10	3.04	0.17	3.44	0.19	3.54	0.56	3.69	0.31	3.13	0.07
13_LM06	4.25	0.37	3.60	0.51	3.69	0.84	3.88	0.46	4.25	0.44	4.25	0.44	4.06	0.54
13_LM07	4.38	0.24	3.88	0.22	3.13	0.77	4.38	0.30	3.75	0.37	4.50	0.29	4.50	0.29
13_LM08	3.25	0.25	2.63	0.22	3.50	0.29	3.13	0.31	3.04	0.24	3.31	0.24	3.50	0.29
13_LM10	4.31	0.45	3.52	0.17	4.56	0.26	3.88	0.46	4.46	0.36	4.13	0.43	3.88	0.43
14_LM01	3.63	0.22	3.17	0.31	3.38	0.48	3.38	0.24	3.31	0.43	3.38	0.30	3.25	0.25
14_LM02	3.63	0.24	3.92	0.28	3.69	0.33	4.13	0.31	4.25	0.23	3.63	0.51	3.81	0.43
14_LM03	3.75	0.78	4.25	0.48	4.13	0.44	4.31	0.34	3.69	0.77	3.88	0.38	4.06	0.54
14_LM05	3.67	0.41	3.67	0.57	3.75	0.60	4.25	0.32	4.19	0.45	4.25	0.48	3.50	0.29
14_LM06	4.19	0.49	4.31	0.45	4.88	0.13	4.31	0.19	4.56	0.26	4.44	0.41	3.75	0.25
14_LM07	4.44	0.33	4.25	0.37	3.56	0.90	4.75	0.25	4.92	0.08	5.00	0.00	4.63	0.13
14_LM08	3.56	0.33	2.88	0.55	4.06	0.41	3.94	0.33	4.25	0.37	3.81	0.62	3.50	0.35
14_LM10	3.75	0.60	4.60	0.21	4.04	0.85	4.06	0.41	4.54	0.18	4.75	0.25	4.31	0.34
07_LM01	3.06	0.36	2.38	0.24	2.94	0.16	2.81	0.19	2.15	0.34	3.50	0.31	2.31	0.31
13_LM02	2.69	0.43	2.75	0.37	2.92	0.40	2.75	0.25	3.25	0.32	2.69	0.28	3.06	0.60
13_LM03	3.25	0.25	3.19	0.19	3.50	0.29	3.44	0.26	2.88	0.41	3.38	0.22	2.88	0.30
14_LM09	2.38	0.30	2.44	0.28	4.19	0.31	2.63	0.24	3.75	0.65	4.00	0.40	3.15	0.46
14_LM13	2.69	0.24	2.56	0.33	2.75	0.37	2.94	0.36	3.40	0.21	3.19	0.19	3.19	0.49
13_LM05	2.56	0.36	1.81	0.19	2.00	0.35	2.25	0.14	2.06	0.16	2.19	0.12	2.44	0.26
14_LM12	2.00	0.00	2.44	0.33	2.13	0.43	3.44	0.36	2.50	0.61	3.00	0.25	3.25	0.25
14_LM11	2.75	0.48	2.81	0.43	3.21	0.43	2.94	0.39	3.13	0.66	3.79	0.31	3.35	0.38
13_LM04	2.13	0.13	2.25	0.18	2.25	0.25	2.38	0.30	2.31	0.19	2.50	0.29	2.50	0.29

	PI167261		PI170622		PI170627		PI175811		PI175812		PI179397		PI223396	
Isolates	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
13_LM09	4.25	0.48	4.19	0.45	4.25	0.48	4.44	0.33	4.50	0.29	4.25	0.48	4.44	0.26
14_LM04	3.88	0.33	4.13	0.43	4.06	0.48	4.06	0.41	3.94	0.41	4.25	0.48	4.44	0.33
07_LM02	3.25	0.25	2.81	0.19	2.94	0.06	3.00	0.00	3.50	0.29	3.06	0.12	3.19	0.28
07_LM03	3.50	0.29	3.56	0.33	3.19	0.28	3.44	0.44	3.63	0.30	3.56	0.63	3.50	0.61
07_LM04	3.38	0.24	3.50	0.23	2.88	0.13	3.50	0.29	3.44	0.52	3.13	0.13	2.75	0.63
07_LM05	3.38	0.58	3.44	0.16	3.25	0.37	3.56	0.50	3.63	0.38	3.69	0.24	4.38	0.46
13_LM06	4.06	0.54	4.31	0.40	4.44	0.40	4.75	0.25	4.31	0.40	4.44	0.48	4.44	0.33
13_LM07	3.44	0.83	4.56	0.21	4.69	0.24	4.75	0.25	4.38	0.24	4.56	0.21	4.69	0.24
13_LM08	3.00	0.41	3.44	0.26	2.88	0.22	3.31	0.24	3.50	0.29	3.50	0.29	3.31	0.24
13_LM10	4.13	0.46	4.38	0.41	4.31	0.34	4.75	0.25	4.50	0.23	4.44	0.48	4.13	0.38
14_LM01	3.31	0.24	3.25	0.25	3.19	0.47	3.38	0.24	3.50	0.29	3.63	0.24	3.75	0.32
14_LM02	3.63	0.47	3.63	0.24	4.25	0.14	3.94	0.33	3.88	0.24	4.31	0.12	3.50	0.50
14_LM03	3.88	0.36	4.19	0.28	3.81	0.47	3.94	0.44	3.88	0.52	4.06	0.41	4.50	0.29
14_LM05	4.25	0.48	4.31	0.40	4.50	0.35	4.60	0.32	4.88	0.07	4.19	0.12	3.88	0.58
14_LM06	4.81	0.19	4.69	0.24	4.63	0.24	4.94	0.06	4.75	0.25	4.38	0.46	4.75	0.25
14_LM07	4.19	0.31	4.75	0.25	5.00	0.00	4.75	0.25	4.19	0.81	4.88	0.13	5.00	0.00
14_LM08	3.50	0.65	4.25	0.48	3.69	0.34	4.25	0.48	4.25	0.48	3.88	0.31	3.94	0.36
14_LM10	4.13	0.59	4.50	0.50	4.06	0.36	4.69	0.24	4.31	0.31	4.38	0.47	4.75	0.25
07_LM01	2.94	0.06	3.44	0.16	3.06	0.39	3.50	0.55	3.44	0.60	3.38	0.46	3.31	0.34
13_LM02	3.25	0.48	3.44	0.28	3.63	0.22	3.50	0.29	3.81	0.12	3.19	0.43	3.21	0.68
13_LM03	3.38	0.24	3.38	0.24	3.19	0.19	3.38	0.24	3.50	0.29	3.31	0.24	3.44	0.26
14_LM09	3.31	0.77	3.31	0.57	3.56	0.48	3.06	0.54	3.94	0.71	2.69	0.51	3.44	0.48
14_LM13	2.94	0.36	3.38	0.60	2.94	0.48	3.38	0.47	3.38	0.47	3.13	0.30	3.33	0.31
13_LM05	2.13	0.41	2.13	0.13	2.44	0.26	2.38	0.24	2.13	0.31	2.44	0.21	2.50	0.23
14_LM12	2.50	0.29	3.00	0.54	3.19	0.45	2.75	0.48	3.25	0.14	2.75	0.43	3.19	0.28
14_LM11	2.75	0.31	2.38	0.22	3.13	0.52	3.00	0.41	3.50	0.29	3.38	0.55	3.69	0.24
13_LM04	2.06	0.06	2.50	0.29	2.50	0.29	2.50	0.29	2.31	0.24	2.56	0.33	2.69	0.19

	PI284184		PI284186		Togo	
Isolates	Mean	SEM	Mean	SEM	Mean	SEM
13_LM09	4.13	0.52	4.38	0.47	4.13	0.52
14_LM04	4.19	0.41	4.31	0.43	3.75	0.27
07_LM02	3.25	0.10	3.13	0.07	3.00	0.00
07_LM03	3.88	0.31	3.88	0.52	3.13	0.13
07_LM04	3.13	0.41	3.69	0.24	3.44	0.52
07_LM05	3.81	0.53	4.13	0.31	3.50	0.54
13_LM06	3.88	0.39	4.75	0.18	3.94	0.54
13_LM07	4.31	0.16	4.31	0.24	4.19	0.45
13_LM08	3.06	0.36	3.44	0.26	3.38	0.24
13_LM10	4.69	0.24	4.44	0.33	3.81	0.36
14_LM01	3.25	0.40	3.81	0.31	3.31	0.28
14_LM02	3.44	0.21	4.06	0.41	3.69	0.21
14_LM03	3.94	0.55	4.13	0.39	4.06	0.48
14_LM05	3.92	0.53	4.38	0.24	3.75	0.67
14_LM06	4.38	0.47	4.75	0.25	4.56	0.36
14_LM07	4.81	0.12	4.88	0.13	4.63	0.24
14_LM08	3.75	0.62	4.06	0.56	3.69	0.34
14_LM10	4.31	0.16	4.25	0.53	4.13	0.39
07_LM01	3.31	0.49	3.19	0.47	2.63	0.30
13_LM02	3.63	0.47	3.25	0.25	3.38	0.30
13_LM03	3.50	0.29	3.44	0.26	3.19	0.19
14_LM09	3.31	0.31	3.63	0.63	3.44	0.71
14_LM13	2.94	0.47	3.63	0.30	2.81	0.41
13_LM05	2.13	0.13	2.40	0.12	2.38	0.22
14_LM12	2.44	0.30	3.25	0.25	2.50	0.29
14_LM11	3.00	0.58	3.38	0.47	2.96	0.53
13_LM04	2.31	0.24	2.63	0.24	2.08	0.22